

2013

Liposomal uptake of silver and gold nanoparticles

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LIPOSOMAL UPTAKE OF SILVER AND GOLD NANOPARTICLES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirement for the degree of
Master of Science in Mechanical Engineering

in

The Department of Mechanical & Industrial Engineering

by

Dipon Chanda

B.Sc., Bangladesh University of Engineering and Technology, 2009

August 2013

Dedicated to my Parents

ACKNOWLEDGEMENTS

First I would like to express my heartfelt gratitude and respect to my supervisor Dr. Ram Devireddy for his continuous support and guidance towards my research work. I would like to thank Dr. Dorel Moldovan and Dr. Ingmar Schoegl for spending their valuable time to serve in my advisory committee and evaluating my thesis.

Special thanks to Dr. Daniel Hayes and his student Ammar Qureshi from Biological and Agricultural Engineering Department for providing the nanoparticles. Special thanks also go to Dr. Ying Xiao from Socolofsky Microscopy Center and Dr. Xiaochu Wu from veterinary school for their help in microscopy.

I would like to thank Diane Morgan, our graduate secretary for her help in all official paperwork throughout my graduate study at LSU. I also thank my colleagues Harish, George and Anoosha at the Bioengineering Lab with whom I spent a memorable time and got help whenever I required.

Finally I would like to mention the name of my friends Sarat Das, Reaj Uddin Ahmed, Rakib Uddin Ahmed and Mostafijur Rahman for motivating me in graduate studies. Thanks to Akik Khan and Imran Akond, Tushar Liakat and Sukanta Bhattacharya, Apu Noor, Iftekhhar Ifti Rouf, Avishek Guha for their help while in LSU.

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ABSTRACT

The main objective of this work is to study the liposomal uptake of silver and gold nanoparticles. Liposomes were prepared in Heating Method. The phospholipids used to prepare liposomes are 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (16:0 PC); 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (16:0 PG); 1,2-distearoyl-*sn*-glycero-3-phosphocholine (18:0 PC); 1,2-distearoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (18:0 PG). Four different combinations of phospholipids were used to prepare liposomes. In all four combinations two types of phospholipids were used. The liposomes were incubated for 30 minute, 1 hour, 2 hour and 4 hour with silver and gold nanoparticles in streptavidin coated glass slide. All four liposomal formulations had a biotinylated lipid (1-oleoyl-2-[12-biotinyl(aminododecanoyl)]-*sn*-glycero-3-phosphocholine) which has a strong binding affinity for streptavidin. As a result biotinylated liposomes were attached to the streptavidin coated glass slide. After incubation the slides were gently washed with water to get rid of unattached nanoparticles and liposomes. Then the slides were examined under microscope to study the uptake. Uptake was calculated as percentage of liposomes showing nanoparticles attached to them out of total number of liposomes. Liposomes prepared from 16PC & 16PG showed highest amount of uptake while 18PC & 18PG liposomes had lowest uptake for both type of nanoparticles. The uptake was slightly higher for the silver nanoparticles for all four types of liposomes than that of gold nanoparticles. Concentration of the silver nanoparticles was almost 25 times higher than the gold nanoparticle concentration. This suggests that the effect of nanoparticle concentration on the uptake was not much strong. In all cases the uptake was nonlinear function of incubation time.

At the beginning the uptake rate was higher which decreased over time. Liposomes prepared from 16PC & 16PG lipids can be great carrier for silver and gold nanoparticle based drug delivery carrier.

1. INTRODUCTION

1.1 Liposomes

Liposomes are artificially prepared vesicles composed of a lipid bilayer. Liposomes were first made in England in 1961 by Alec D. Bangham [1, 2]. Amphiphilic phospholipid molecule in aqueous media can arrange themselves to make a spherical vesicular structure which is called Liposome. Phospholipid molecules have both hydrophilic and hydrophobic sections within themselves. As a result they can absorb and release two materials with different solubility simultaneously [1]. There are various types of liposomes such as unilamellar, multilamellar and multi vesicular. Their size ranges from twenty nanometers to few micrometers [1].

1.2 Composition of liposomes

Liposomes are mainly prepared from different types of phospholipids or combination of phospholipids. Cholesterol is used to improve membrane property. Poly ethylene glycol (PEG) improves the circulation properties such as longer circulating time [1, 2]

1.3 Preparation Methods

Several methods have been developed by the researchers for the preparation of Liposomes. All of them have their own advantages and disadvantages [1- 3]. They are discussed in brief below.

1.3.1 Bangham Method

Bangham was the first person to prepare liposomes. His method of preparation is known as Bangham Method. This method is also known as thin film hydration method. First the

constituent lipids are dissolved in an organic solvent and taken on a round bottom flux. Next step is the removal of solvent from the solution usually via evaporation to make a thin dry lipid film at the bottom of the flux. The evaporation can be done under nitrogen stream to create an inert atmosphere and to prevent oxidation of phospholipids. Then the thin lipid film is hydrated by adding some aqueous media along with vortexing to prepare multi lamellar vesicles [1-3]. The MLV suspension can be sonicated to prepare large unilamellar vesicles [4]. The hydration and sonication steps are carried out above the phase transition temperature of the phospholipids used in the preparation [1-4].

MLV can be prepared easily in this method. The liposomes produced are heterogeneous in size distribution. Encapsulation efficiency is low. Organic solvent cannot be removed fully. Sterilization of liposomes is another issue to consider [1-3].

1.3.2 Detergent Depletion Method

Lipid can be hydrated with detergent solution to produce detergent-lipid micelles. Also both lipid and detergent can be dried from an organic solution and then the hydration can be done to make detergent-lipid micelles. Next step is the removal of detergents to produce liposomes. The detergent is removed by dilution, dialysis, and adsorption or column chromatography. Among the methods dilution is the simplest. The liposome produced in this method is homogeneous in nature. The rate of detergent removal from the solution and initial ratio of detergent to phospholipids determines the size and homogeneity of the liposomes produced in this method [1, 2].

The concentration of liposomes at final stage is low. Encapsulation of any hydrophobic compound is also low. The method requires much time to produce liposomes. Also some hydrophilic compounds may be removed during the detergent removal step [1, 2].

1.3.3 Injection Method

In ether injection method first the constituent lipids are dissolved in ether and the solution is injected into an aqueous media which is kept at a temperature higher than the boiling temperature of ether. The ether evaporates upon contact with the aqueous media as the temperature is high and the lipids form mainly unilamellar liposomes. Millipore filtration can remove multilamellar vesicles and aggregates and gel filtration can remove the residual ether from the liposomal suspension [5].

The process is fast and takes less than an hour. Entrapment efficiency is higher compared to that of sonicated liposomes. But the liposomes are heterogeneous in size distribution. Some lipids are lost in the filtration process. Aggregation is a problem for uncharged liposomes. Compounds which are sensitive to ether are not suitable to be encapsulated in this method [5].

In ethanol injection method lipids are first dissolved in ethanol and the solution is then injected on an aqueous phase to prepare liposomes. The method is simple, first and reproducible. Some lipids have low solubility in ethanol and the liposomes are heterogeneous if mixing of the phospholipid is not adequate [1, 2].

In a variation of the ethanol injection method inkjet printer were used to inject a phospholipid solution in ethanol into water to produce unilamellar liposomes in the size range of 50 to 200 nm. The method was reproducible [6].

1.3.4 Reverse Phase Evaporation Method

In this method first the phospholipids are dissolved in an organic solvent. Then an aqueous phase is introduced into the solution. Next step is the removal of organic solvent by evaporation under reduced pressure to prepare liposomes. Chromatography, dialysis or centrifugation can be used to remove unencapsulated compound and residual organic solvent after the preparation. The method is simple and encapsulation efficiency is high. [7].

The method is time consuming. Another disadvantage of the method is the contact of organic solvent with the compound to be encapsulated and so the method is not suitable for encapsulating some fragile compounds [1, 2, 7].

1.3.5 Microfluidic Channel Method

In this method a lipid stream dissolved in alcohol is passed in between two aqueous streams in a microfluidic channel. Laminar flow condition is maintained in the channel which enables controlled diffusive mixing at the two liquid interface and liposomes are produced as a result. The size of the liposomes is a function of the ratio of the alcohol to aqueous phase volume flow rate. Liposomes of mean diameter from 50 to 150 nm were prepared by adjusting the ration of the alcohol to aqueous volumetric flow rate. The method can produce liposomes in reproducible manner [8].

1.3.6 Supercritical Reverse Phase Evaporation Method

First phospholipids and organic solvent are taken into a variable volume cell. Then CO₂ is introduced into the cell. The temperature is increased above the phase transition temperature of the phospholipids. Pressure is increased by decreasing the volume of the cell. An aqueous

solution containing the compound to be encapsulated is injected into the cell by using a pump. The cell component is stirred using a magnetic stirrer. Then the CO₂ is removed from the cell and a uniform liposome suspension is obtained [9, 10]. The entrapment efficiency of this method is higher than Bangham method for water soluble compounds. Liposomes prepared from unsaturated phospholipids having unsaturated alkyl chain showed higher entrapment efficiency than that of liposomes prepared from phospholipids with saturated alkyl chain. Liposomes prepared from phospholipids having both saturated and unsaturated alkyl groups showed some value in between. The optimal alkyl chain length was C₁₆ and C₁₈. Higher or lower alkyl chain length adverse effect on entrapment efficiency or decreased the osmotic response of liposomal membrane [9].

A modified version of the above method is Improved Supercritical Reverse Phase Evaporation Method where no organic solvent is used. The phospholipids and aqueous solution are taken on a cell. Cell temperature is increased above the phase transition temperature of the phospholipids and CO₂ is introduced into the cell. Pressure is raised up to 200 bar. Then the cell components are stirred for a definite period of time. Then the CO₂ is released to produce liposomes. In an experiment entrapment efficiency of glucose was higher than that of Bangham method. Liposomes prepared in this method were stable after one month of preparation at room temperature [11].

1.3.7 High Pressure Homogenization Method

In this method high pressure homogenizer is used to process liposomes prepared from other methods. At high pressure the structure of liposomes is disrupted and rearrangement of liposome structure takes place due to various forces [1]. The mean diameter of the resulting

liposomes is a function of both the inlet pressure and the number of processing cycles. The mean diameter decreases with increasing inlet pressure and number of cycles. The entrapped volume shows a similar pattern to the mean diameter. On the other hand the mean diameter is increased with increasing ionic strength of the aqueous medium [12].

The method is simple and can be used for bulk production. The drawbacks are sterilization issue, required high pressure and non-homogeneous liposomes preparation [1].

1.3.8 Extrusion Method

In this method unilamellar liposomes can be produced by extruding multilamellar vesicles through polycarbonate membrane of desired pore size at elevated pressure [13]. Homogeneous unilamellar liposomes can be obtained using filters with nominal pore diameter ranging from 30 to 400 nm [14]. Freeze-thaw cycles can be applied along with the extrusion. Freezing and thawing prior to extrusion significantly increases the unilamellarity and entrapment efficiency of the liposomes [13]. The process is suitable for very high lipid concentration up to 400 mg/ml [14]. Multilamellar vesicles are produced by hydrating dry lipid film in appropriate aqueous buffer along with vortexing. The extrusion is done at a temperature above the phase transition temperature of the constituent lipids [13, 14]. Liposomes made in extrusion method have been used to encapsulate a magnetic resonance contrast agent and an X-ray contrast agent iopromide simultaneously. The liposomes were radiopaque and paramagnetic [15].

The method does not require the use of organic solvents or detergents. High concentration of lipids can be used with high entrapment efficiencies. Also the process is rapid [13].

1.3.9 Dual Asymmetric Centrifugation

This is a special technique to prepare liposomes. In the method regular centrifugation is carried out along with a second rotation of the sample around its own vertical axis. This extra rotation creates shear forces in the sample and results in efficient homogenization. First highly concentrated blend of hydrogenated phosphatidylcholine, cholesterol and NaCl was centrifuged to prepare viscous vesicular phospholipid gel (VGA) which is subsequently diluted to prepare liposomes. The factors affecting the liposomal size are centrifugation speed, concentration of lipids, and time of homogenization and use of mixing aid like glass beads. The method was reproducible under optimum condition and small liposomes in the size of 60 ± 5 nm can be produced [16]. Liposomes prepared in dual asymmetric centrifugation method have been used to encapsulate siRNA in sterile condition with high entrapment efficiency. The siRNA was intact during the centrifugation process. The liposomal formulation was stable for at least 3 months [17].

The method is simple and rapid with high entrapment efficiency. It is not suitable for bulk production [16, 17].

1.3.10 Heating Method

In this method the lipids are first hydrated at room temperature for about one to two hour. Then they are mixed at high temperature (up to 120°C) in the presence of glycerol. The resulting suspension is then extruded through polycarbonate filters of desired pore diameter to prepare liposomes. The extrusion is done above the phase transition temperature of the phospholipids [18-20]. No degradation of the lipids was observed at high temperature. Liposomes were stable after eight months of their preparation and storage under nitrogen at 4°C

. Liposomes prepared in heating method have been used to prepare stable anionic liposome-plasmid particles [19]. They have also been used to prepare liposomal gene therapy vector. The liposomal formulation was stable in long term. It also showed high DNA entrapment efficiency and high transfection efficiency [20].

The method is simple and free of any organic solvent. As a result of heating no extra sterilization procedure is required [18-20].

1.3.11 Special Method

Preparation of liposomes has been reported by changing the pH of the aqueous media rapidly followed by a period of equilibrium. First the lipids are hydrated in an aqueous buffer along with vortexing at a pre-defined temperature. Then the pH of the medium is suddenly increased to 11 by using NaOH and then adjusted to pH 7.4 by using HCl. The time period of pH adjusting is termed as pH jumping time. Then the solution is left for a fixed amount of time termed as period of equilibrium. At this point the heating and stirring is stopped. Then the solution is left to cool to room temperature. Then centrifugation was carried out to eliminate aggregation and larger sized liposomes. The factors affecting the liposomal size, shape and monodispersity are time interval of pH jump, operation temperature, equilibrium time and type of the phospholipids used [21].

1.4 Applications

Liposomes have versatile applications due to their unique properties [1]. Some applications are discussed below in brief.

1.4.1 Cosmetics

Natural marine lipid extract containing a high polyunsaturated fatty acid ratio have been used to prepare liposomes in a condition that mimic that of topical application in terms of temperature, calcium and pH. The toxicology file of the liposomes showed a good eye and skin tolerance. As a result these liposomes have a high potential for application in cosmetics [22].

Liposomes made from egg and soya phospholipids have been used to incorporate a moisturizing cream and applied to skin to study the effect of liposomes. Comparison was made among the moisturizing cream and four liposomal formulations of the cream. Liposomes made from egg phospholipids showed better results than liposomes prepared from soya phospholipids in terms of skin water content [23].

1.4.2 Food Science

Encapsulating Nisin Z in liposomes can improve Nisin stability and inhibitory action in cheddar cheese. Encapsulation also protects the cheese starter from the adverse effect of Nisin during cheese production. Thus encapsulating Nisin Z in liposomes can eliminate the adverse effects of using free Nisin in cheddar cheese [24].

Incorporation of lipase encapsulated liposomes in cheddar cheese accelerated lipolysis. The treated cheese showed higher moisture and lower fat, protein and ash content than the control cheese. The treated cheese was less firm but more elastic and cohesive. Addition of encapsulated enzyme accelerated the production of free fatty acid [25].

Use of liposomes encapsulating enzymes can be used to accelerate cheese ripening. Proteinase from bacteria and fungus was encapsulated in liposomes and used during cheese

ripening. Experimental cheese had higher moisture but lower protein content. Treated cheese was less firm but more brittle than control cheese. Proteolysis and texture development of experimental cheese were faster than control cheese [26].

Encapsulating ferrous glycinate into liposomes improved the stability of the compound in strong acid environment simulating gastrointestinal juice. Thus ferrous glycinate liposomes have high potential as iron fortifier [27].

Ferrous sulphate has been incorporated in liposomes and added to milk to increase the iron concentration. The formulation was stable upon heating at 100°C for 30 minute and storage at 4°C during one week [28].

Absorption of α -Tokopherol in lymph was three times increased by encapsulating the compound inside marine lipid based liposomes [29].

Encapsulation of β -galactosidase inside liposomes can reduce the hydrolysis of lactose when used in milk prior to consumption. This helps the retention of original milk flavor and the digestion of lactose after consumption [30].

Slaughter blood haem has been encapsulated into liposomes and used for the fortification of bread dough. The addition of liposomal haem increased the fat content of flours. It also had positive effect on the stability of the dough. Loaf volume and uniformity of crumb was also improved [31].

1.4.3 Pharmaceutical and Medical Research

Nisin loaded Nanoliposomes prepared by Mozafari method have been incubated with bacteria and microscopy showed the accumulation of liposomes around the bacteria. Also fusion of some liposomes with the bacterial membrane after two hours of incubation at 37°C was observed. The liposomal formulations were stable for at least 14 months at 4°C and for 12 months at 25 °C [32].

Liposomes prepared with and without polyethylene glycol (PEG) have been used to encapsulate albendazole, a hydrophobic drug used for the treatment of hydatid cysts. In vitro drug release study showed that the PEGylated liposomes released drug over a longer period of time than conventional liposomes. The encapsulation efficiency was also higher for PEGylated liposomes [33].

Cholesterol succinyl chitosan anchored liposomes have been used to encapsulate epirubicin, an anticancer drug having wide range of antitumor activity. In vitro study showed more sustained release of drug in both phosphate buffer solution (pH 7.4) and 1% (vol/vol) fetal bovine serum compared to plain liposomes. The stability was also higher compared to plain liposomes [34].

The basic fibroblast growth factor (bFgF) has a very short half-life in vivo which limits its therapeutic use. Encapsulating it with liposomes can improve the stability and prolong its effects in vivo. Studies with rat shows that bFgF encapsulated with liposomes had better effect for wound healing compared with bFgF alone [35].

Liposomes encapsulating amikacin sulphate (AMK) have been used to prepare dry powder inhaler formulations. Encapsulating the drug inside liposomes prolonged the release of drug at the localized site. This reduced the side effects of the drug and frequency of dosing [36].

Liposomal formulations have been prepared encapsulating recombinant human insulin along with sodium glycocholate for oral insulin delivery. Sodium glycocholate acts as an enzyme inhibitor and permeation enhancer. In vitro study of the formulation showed protection of insulin against enzymatic degradation by trypsin, pepsin and α -chymotrypsin [37].

Boron neutron capture therapy is a method for the treatment of cancer which requires the selective accumulation of boron in tumor tissue. Transferrin conjugated polyethylene glycol liposomes have been used to encapsulate drugs containing boron. In vivo studies with rat showed enhanced and prolonged accumulation of boron compound into the solid tumor tissue. The long term survival rate was higher for the transferrin polyethylene glycol (PEG) liposomal formulation compare to PEG liposomes, plain liposomes and free drug [38].

Paclitaxel is an approved drug for the treatment of ovarian and breast cancer. Conventional commercial formulation is associated with hypersensitivity reactions. Liposomal formulation have been made and tested with mice which showed well tolerance and efficient treatment of cancer cells [39].

Liposome surface coated with polymer having a hydrophobic moiety have been used to encapsulate doxorubicin, an anticancer drug. Studies with rat showed targeting efficiency of polymer coated liposomes were five times higher than the non-coated liposomes [40].

Liposomal formulation containing tamoxifen citrate has been prepared for the sustained release of the drug. In vitro studies showed the 50% release of drug within three hours and 95% within thirty hours. Liposomes stored at 2-8°C were most stable and only 4% of drug was lost over the storage period of five weeks [41].

Octadecyl quaternized carboxymethyl chitosan can be used as a liposomal ingredient. These liposomes have increased physical and thermal stability, higher solubility in water and increased drug encapsulation efficiency. These polymer modified liposomes have been used to encapsulate vincristine. The liposomal formulation was stable in aqueous solution and showed slow and steady release of drug over two weeks [42].

Charged and neutral liposomes have been used to encapsulate four types of aminoglycosides. Cationic liposomes showed highest entrapment of aminoglycosides. Studies showed intracellular killing of *Staphylococcus aureus* by canine monocytes was increased when liposomes containing aminoglycosides were added to the cultures. The liposomal formulation was not toxic to the canine monocytes. The phagocytic ability was not decreased by the liposomal formulation [43].

pH sensitive fusogenic Liposomes modified with succinylated polyglycidol encapsulating anti BCG monoclonal antibodies have been used to target tumor cells. In vitro studies with mouse colon carcinoma 26 cells showed liposomes attached to the cells and endocytosis occurred at 37°C. Endocytosis phenomena occurred in under acidic conditions. Mouse colon carcinoma 26 cells share a common antigen with BCG. The liposomes recognized the target cells and transferred their content into the cytoplasm by fusion with cell membrane and subsequent internalization into the cells by endocytosis [44].

Liposomes have been used to make a laser triggered drug delivery carrier system. Liposomes encapsulating 6-carboxyfluorescein and dextran-magnetite have been used for this purpose. Significant amount of carboxyfluorescein was released in response to a nanosecond laser pulse. The probable reason is the increase of liposomal temperature due to laser exposure of magnetic materials [45].

Temperature sensitive drug delivery system has been made by modifying liposomes surface with a temperature sensitive polymer. Purpose was to increase the degree of drug release from liposomes at the hyper thermic temperature. Calcein was used as a model drug. The release behavior of Calcein was a function of both temperature and time. The temperature sensitive polymer used was N-isopropyl acrylamide [46].

Liposomes have also been used to make an ultraviolet ray induced drug delivery carrier system. Photoactive destabilization agent was suprofen. Calcein was used as a model drug. Release of Calcein upon exposure to ultraviolet ray was higher for suprofen containing liposomes than that of plain liposomes. Linear relationship was found between ultraviolet exposure and amount of Calcein released [47].

Liposomes are also used as gene delivery vector. Cationic liposomes are usually employed for this purpose. Incorporating magnetic particles in the liposomes can make them as a magnetic carrier system. Magnetic field is used for the accumulation of liposomes in the target tissue. Transfection efficiency was higher for magnetic liposomes compared to plain cationic liposomes. These liposomes can be used an efficient gene delivery vector [48].

2. MATERIALS AND METHODS

2.1 Preparation of Liposomes

There are several methods for the preparation of liposomes in small and large scale which have their own advantages and disadvantages [1-3]. Heating Method developed by Mozafari et al can produce sterile, non-toxic liposomes without use of any organic solvent [18]. The method requires the hydration of constituting phospholipids followed by heating in the presence of glycerol above the phase transition temperature of the phospholipids [18-20]. Mozafari reported that in the presence of cholesterol liposomes were prepared at 120°C and without cholesterol they can be prepared at 60-70°C which is greater than the phase transition temperature of most phospholipids [18]. In this study Liposomes were prepared in Heating Method without the use of any cholesterol and the heating was done at about 100°C . Four different combinations of phospholipids were used to prepare liposomes. The phospholipids used to prepare liposomes are 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (16:0 PC); 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (16:0 PG); 1,2-distearoyl-*sn*-glycero-3-phosphocholine (18:0 PC); 1,2-distearoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (18:0 PG). All the phospholipids were bought from Avanti Polar Lipids Inc. and stored in the freezer. In all four combinations two types of phospholipids were used. First the constituent phospholipids were measured in a measuring scale (Scientech) and taken in a small vial. Before opening the lid of the phospholipids bottle it was kept in open air for some time so that the lipid temperature becomes almost equal to the air temperature. Otherwise vapor can condense inside the phospholipid bottles and that will degrade the lipids in the long run. In all formulations the lipids were used in 1:1 molar ratio. Then 1mg of Biotin PC (1-oleoyl-2-[12-biotinyl(aminododecanoyl)]-*sn*-glycero-3-phosphocholine) was added to the vial. Biotin has a strong binding affinity for avidin and that

was used to attach the liposomes having biotinylated lipid on a streptavidin coated glass slide. Then PBS (1x) was added to the vial to make the final concentration of lipids about 2.5 μ mol/ml. Then the lead of the vial was closed. After that the vial was hold on a vortex generator (Scientific Industries Inc.) for 2/3 minutes for the mixing of the phospholipids with the PBS. Then the vial was kept at room temperatures for one hour. This was done so that the hydration of phospholipids can take place. In the meantime water was taken on a Pyrex beaker (250 mL) and the beaker was placed on a hot plate stirrer to make boiling water. Then the vial was put on the boiling water beaker. It was kept at boiling water beaker for another one hour. After that the heating was stopped and the vial was taken out of the beaker. The lipid suspension in the vial was taken on a glass syringe (250 μ L). The syringe was part of the liposome extruder set (Avanti Polar Lipids Inc.) and it was completely air tight. The suspension was then extruded through polycarbonate membrane of 100 nm/1 μ m pore diameter to prepare liposomes. The lipid suspension was extruded through the filter fifteen times. Although it was mentioned in the manual that eleven times pass is enough to get a homogeneous size distribution. Initially liposomes were prepared and characterized using filters of 100nm diameter. Later all the experiments were done using liposomes prepared by extrusion through 1 μ m nominal pore diameter. Liposomes prepared in the size of 100 nm can be characterized by electron microscopy but hard to see and analyze by confocal microscopy. Larger liposomes prepared by extruding through polycarbonate filters of 1 μ m pore diameter were studied using Confocal Laser Scanning Microscopy (CLSM) and Transmission Light Microscopy.

2.2 Characterization

Electron Microscopy is often used to determine the size and shape of liposomes [1]. Scanning Electron Microscopy (SEM) has been previously used to characterize liposomes [49]. Transmission Electron Microscopy (TEM) was also used to visualize liposomes [50]. Cryo-TEM micrograph and Freeze fracture electron micrograph are other two methods which can be used for the characterization of liposomes [51, 52]. In this study SEM and TEM were done to characterize the liposomes of 100 nm size. Laser Scanning Confocal Microscopy and Transmission Light Microscopy were used to study liposomes prepared by extrusion through 1 μm nominal pore diameter. All the Electron Microscopy was done in the Socolofsky Microscopy Center under the department of Biological Science. The model of Scanning Electron Microscope was JSM-6610, JSM-6610LV. The model of the Transmission Electron Microscope was JEOL 100CX. The liposomes were homogeneous in size and almost circular in shape. SEM was done first for one sample without extrusion through the polycarbonate membrane and the liposomes observed were not homogeneous. Then TEM was done for another sample which was extruded through polycarbonate membrane of 100nm. The Liposomes observed were almost of circular shape and size distribution was homogeneous. Later the liposomes were made by extrusion through polycarbonate membrane of 1 μm nominal pore diameter. These liposomes were studied using Laser Scanning Confocal Microscopy and Transmission Light Microscopy. The microscopy was done using Leica TCS SP2 Confocal Laser Scanning Microscope. The 1 μm liposomes were easily visible under light microscopy and the optical resolution of the microscope was good enough to distinguish liposomes separately. Light microscopy images were taken using HCX PL APO CS 40x1.25 and HCX PL APO CS 63x1.25 oil lens.

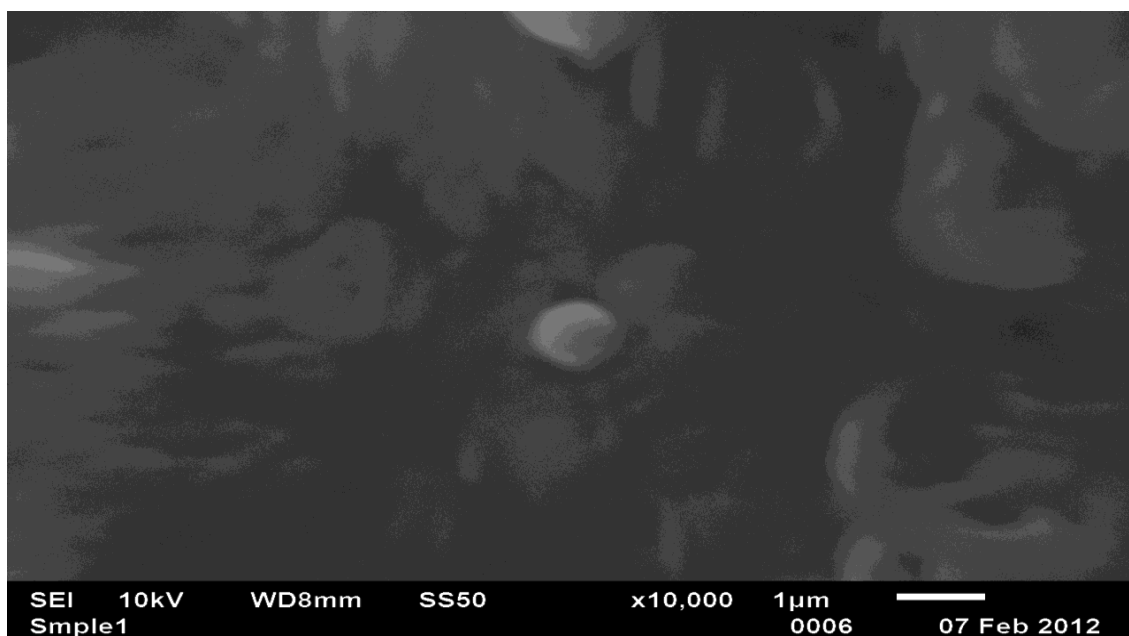
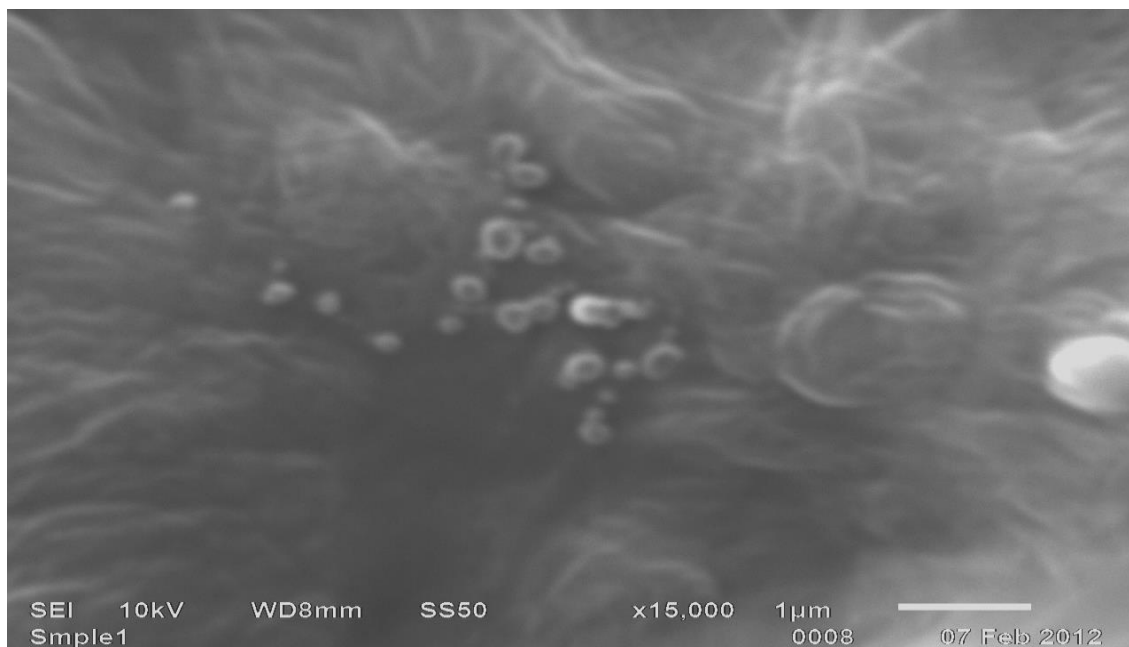
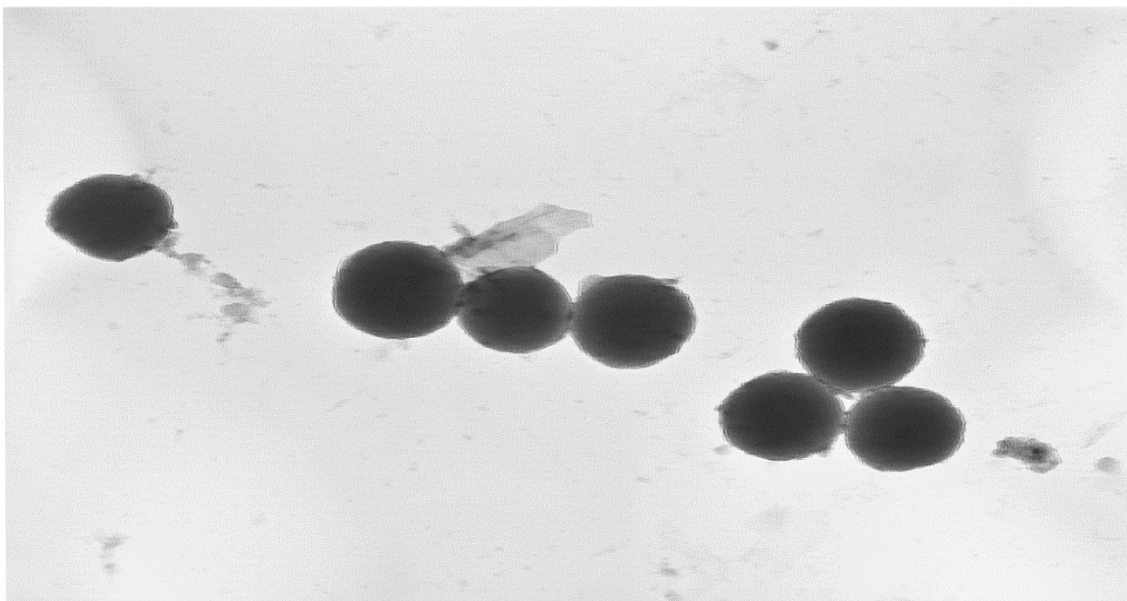


Fig 2.1 SEM Image of Liposomes

0.25 μ m



100 nm

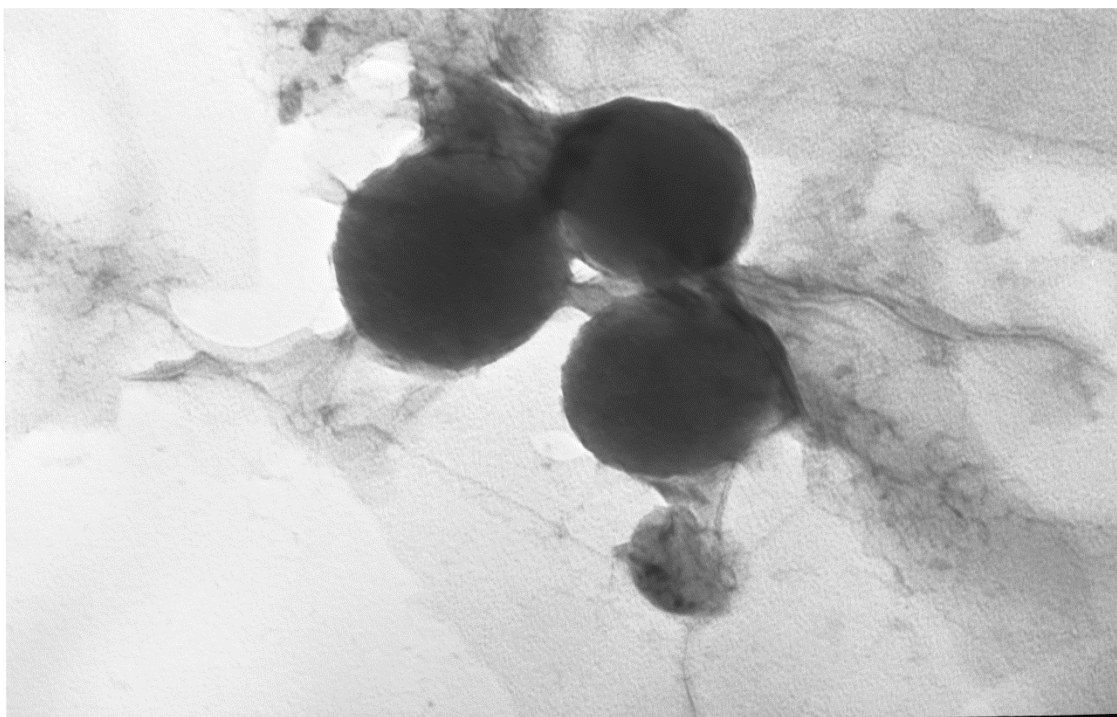


Fig 2.2 TEM Images of Liposomes

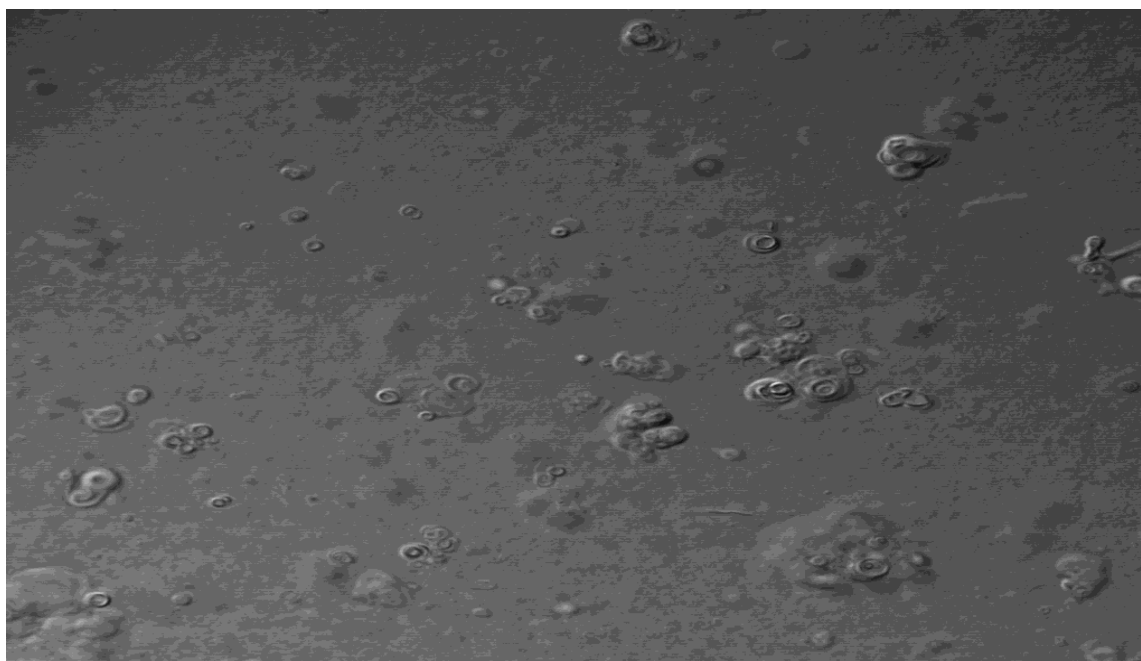
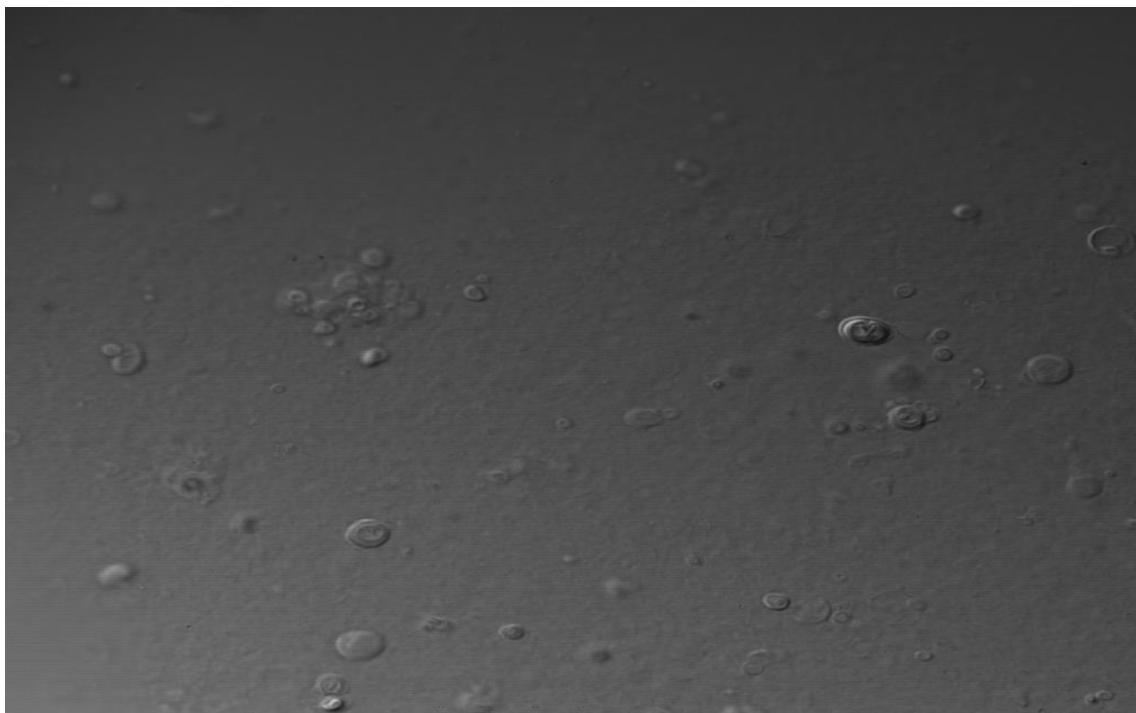


Fig 2.3 Light Microscopy of Liposomes (Transmission Image)

2.3 Attaching Liposomes on Glass Slides

Cells attach to glass surfaces while cultured and can be studied easily under microscope. On the other hand it requires special technique to attach liposomes on glass surface. Researchers have tried for years to attach liposomes on solid surfaces which have various applications. Egg Phosphatidylcholine liposomes have been immobilized successfully on chemically modified hydrophobized Sephacryl gel. The liposomes were also detachable from the gel [53]. Liposomes having biotin group were successfully attached to avidin gels by mixing. The liposomes remained stable after storage and chromatography [54]. Liposomes having PEG-triphenylphosphine have been attached to azide modified glass slide by Staudinger ligation. The immobilized liposomes remained intact [55]. Microwells etched on a silicon substrate array and functionalized with aminosilane-biotin-neutravidin were used to attach liposomes having biotinylated lipids. The liposomes remained stable and intact [56]. Gold surface coated with avidin have been used to attach liposomes having biotinylated lipids. Higher biotin concentration showed greater attachment and at biotin concentration higher than 30mol% the rupture of vesicle was observed forming planer lipid bilayers [57]. Avidin Biotin binding is a popular and very effective method to attach liposomes on solid surfaces. Usually the solid surface is coated with avidin and liposomes are made with biotin functionalized phospholipids. Biotin-Streptavidin binding have been used for this purpose [58-61]. Biotin-Neutravidin binding have also been used [62, 63]. In this study streptavidin coated glass slide was used to bind liposomes having biotinylated lipids.



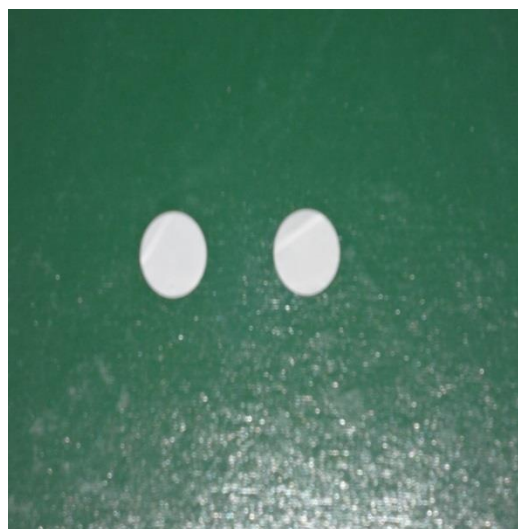
A



B



C



D

Fig 2.4 Apparatus: A is extruder set with heating block. B is air tight syringe. C is extruder set with syringe attached. D is polycarbonate membrane.



E



F



G

Fig 2.5 Equipment: E is vortex generator. F is electronic measuring scale. G is hot plate stirrer

2.4 Nanoparticle Uptake Experiments

Liposomes were prepared using four different combinations of phospholipids. For all set of liposomes experiments were done for four different time of incubation and two different types of nanoparticles. The times of incubation were thirty minute, one hour, two hours and four hours.

Silver and gold Nanoparticles were used for the experiments. Nanoparticles were collected from Dr. Daniel Hayes from the department of Biological and Agricultural Engineering. Silver Nanoparticles were collected in powder form. First 5mg of nanoparticles were measured in a measuring scale and taken on a 10ml test tube. Then 5ml distilled water was added. At this point the concentration of the nanoparticles was 1mg/ml. Then the suspension was divided into two parts. One part was kept as it was and the other part was diluted adding more water and the concentration was made 0.5mg/ml. The same procedure was done for the second sample and the third sample had a concentration of 0.25mg/ml. The third sample was used for all the experiments. The Gold Nanoparticles were collected as solution in water and the concentration was 9.53 +/- 0.92 mg/L. The average size of the nanoparticles was 60-80 nm.

For the experiments of thirty minute incubation the procedures are as follows. First forty μ L of liposomes were measured in a measuring pipette and poured on a streptavidin coated glass slide. It was kept in open air for thirty minutes. Then ten μ L of nanoparticle suspension was added to the liposomes. Then the slide with liposomes and nanoparticles was kept in open air for another thirty minutes. Then the slide was gently washed with water to wash off extra

nanoparticle and unattached liposomes. Later the slide was watched under microscope to determine the uptake of nanoparticles by the liposomes.

For one hour incubation time first forty μL liposomes was poured on a streptavidin coated glass slide and then ten μL nanoparticle suspension was added on top of the liposomes. Then the slide was kept at open air for one hour. The slides were then gently washed with water. After that the slides were studied under microscope.

For two hour incubation first forty μL Liposomes and ten μL Nanoparticles suspension were taken on a vial and the lid of the vial was closed. After one hour the mixed solution was transferred from the vial on a streptavidin coated glass slide by pipette. Then the slide was kept on open air at room temperature for one hour. Then the slide was gently washed with water. It was later examined under microscope.

For four hour incubation first forty μL Liposomes and ten μL Nanoparticles suspension were taken on a vial and the lid was closed. After three hours the mixed solution was transferred on a streptavidin coated glass slide from the vial by pipette. Then the slide was kept on open air for one hour. Then the slide was gently washed with water. Later the slide was examined under microscope to determine the amount of nanoparticle uptake.

By doing the above procedure all the samples were exposed to air for same time period of time (One hour). At the first stage of experiments samples dried due to evaporation which were exposed to air for more than two hours. Then this procedure was followed to overcome the difficulty.

2.5 Microscopy

Nanoparticles made from noble metals can be used for imaging purposes using their property of reflecting/scattering light. Gold Nanoparticles conjugated with mouse monoclonal antibody have previously been used in Laser Scanning Confocal Microscopy to image SKBR3 breast carcinoma cells using reflection/scattering of light by the nanoparticles [64]. Compound cellular imaging has been done using the scattered light of gold nanoparticles and fluorescence of Propidium Iodide (PI) to image human breast cancer cell line [65]. Scattering of light has also been used to detect antisense drug made using silver nanoparticles decorated with thiol-terminated photolabile DNA oligonucleotides inside cells along with fluorescence microscopy [66]. Gold nanoparticle conjugated with anti-epidermal growth factor receptor antibodies have been used to image cancer cells using both reflection and transmission of light [67].

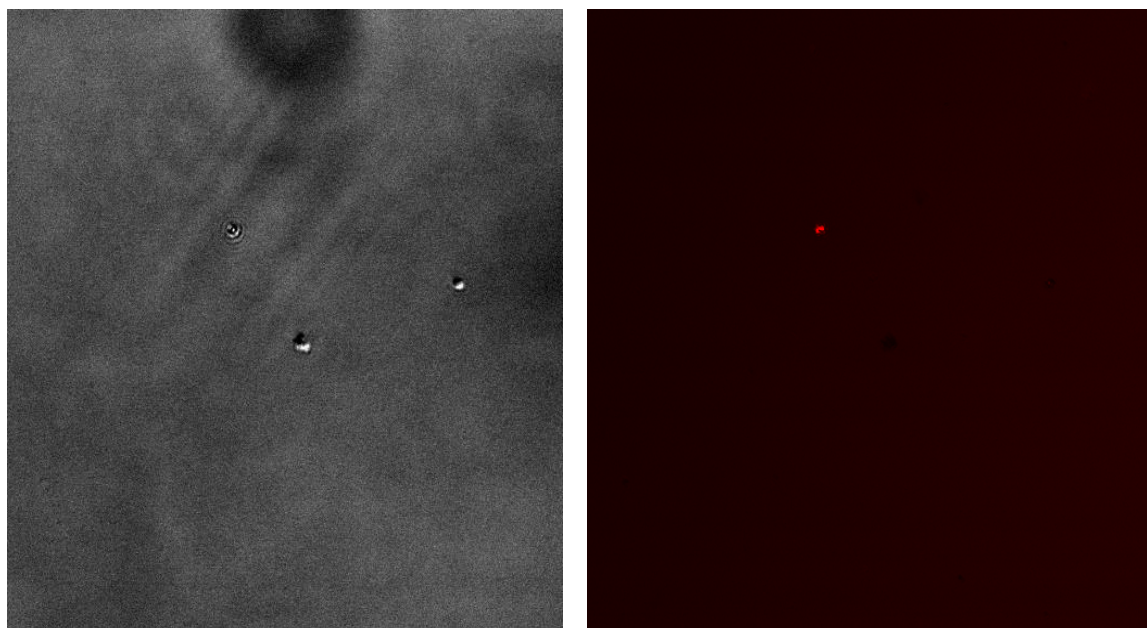
In this study we examined the reflection/scattering of light by nanoparticles (Silver/Gold) to detect them inside liposomes. The aim of the experiments was to study the uptake of nanoparticles by the liposome. In this study both reflection and transmission modes of imaging were used. All the microscopy was done using a Leica TCS SP2 Confocal Laser Scanning Microscope. An Argon laser was used for the reflection images. The excitation was at 488 nm wavelength. Reflection and transmission images were taken simultaneously. HCX PL APO CS 40x1.25 and HCX PL APO CS 63x1.25 oil immersion lens were used. The pseudo color scheme for transmission images were selected as grey and the pseudo color scheme for the reflection images were selected as red. Images were taken on a single focal plane.

In reflection mode a laser light of a defined wavelength is passed through the sample. The reflected light is captured and image is formed which can be analyzed by a software.

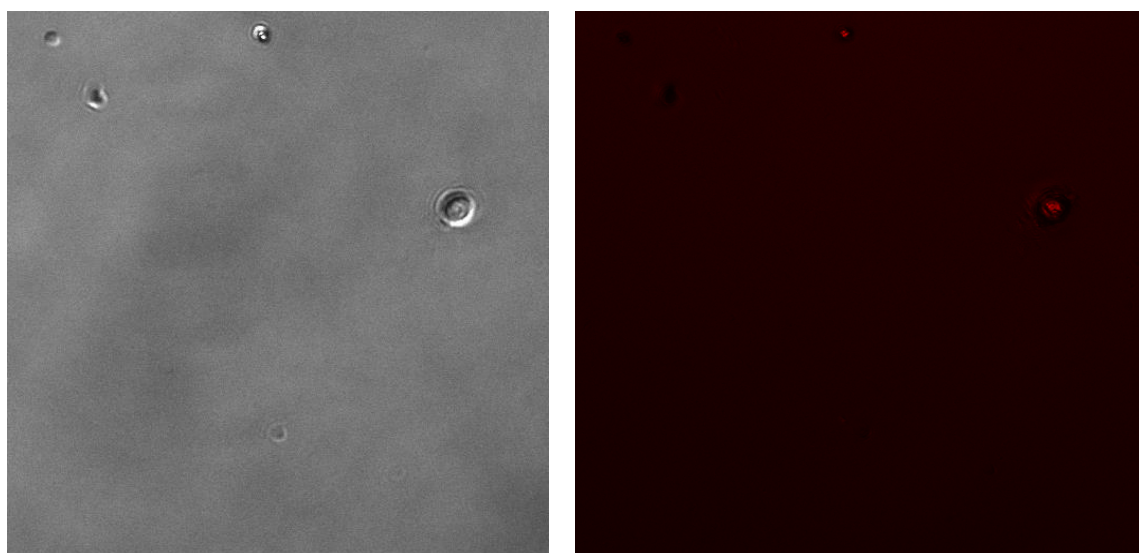
In Transmission mode light is passed through the sample and some light is transmitted. The image is formed from the transmitted light.

2.6 Image Analysis

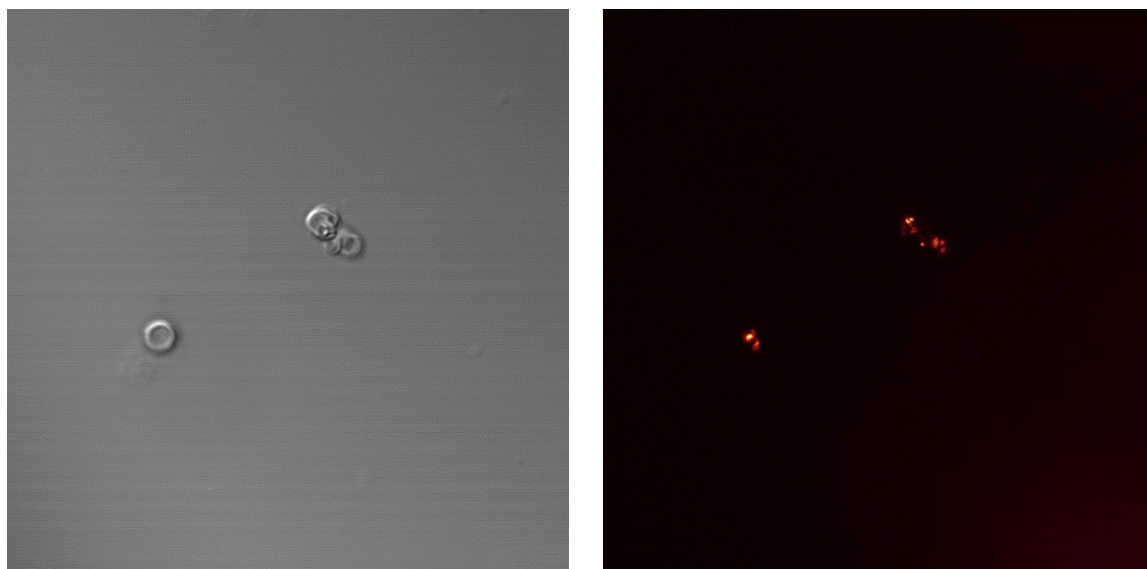
All the images were taken in both transmittance and reflectance mode of the Microscope. The liposomes are visible in transmittance mode and the Nanoparticles reflect light so they are visible on reflectance mode. Liposomes which have absorbed or up taken Nanoparticles will reflect some light in reflectance mode. So comparing the images of reflectance and Transmittance mode one can determine the number of liposomes showing uptake out of total number of Liposomes. The uptake was calculated as a percentage of total number of liposomes. Total numbers of Liposomes were calculated from the image taken in transmittance mode and the number of liposomes showing Nanoparticle uptake was determined from the reflectance mode of the microscopy. Although the resolution of the microscope was not capable of detecting a single nanoparticle the images were clear enough to tell whether there was any uptake. Some sample images are given below showing calculation of uptake.



A
(1 liposome showing uptake out of 3)

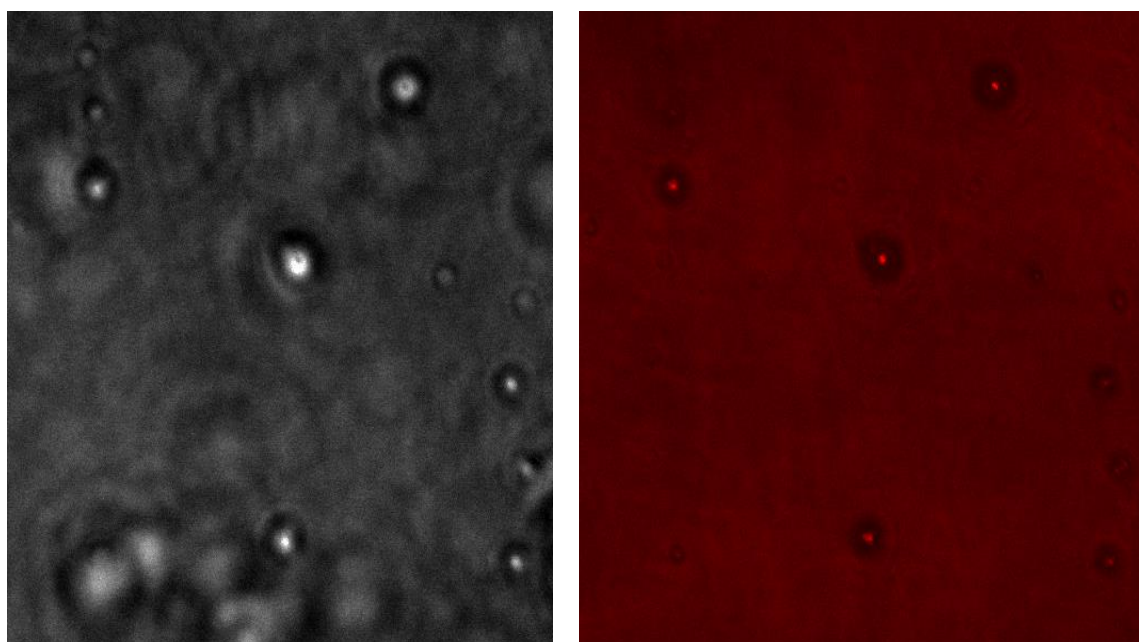


B
(2 liposomes showing uptake out of 5)
Fig 2.6 (A-B) Calculation of uptake.



C

(4 liposomes showing uptake out of 4)



D

(4 liposomes showing uptake out of 11)

Fig 2.6 (C-D) Calculation of uptake

3. RESULTS AND DISCUSSION

Results are presented as a percentage of liposomes showing nanoparticle uptake out of total number of liposomes. Relative comparison can be made among four different liposomal formulations and two different types of nanoparticles used.

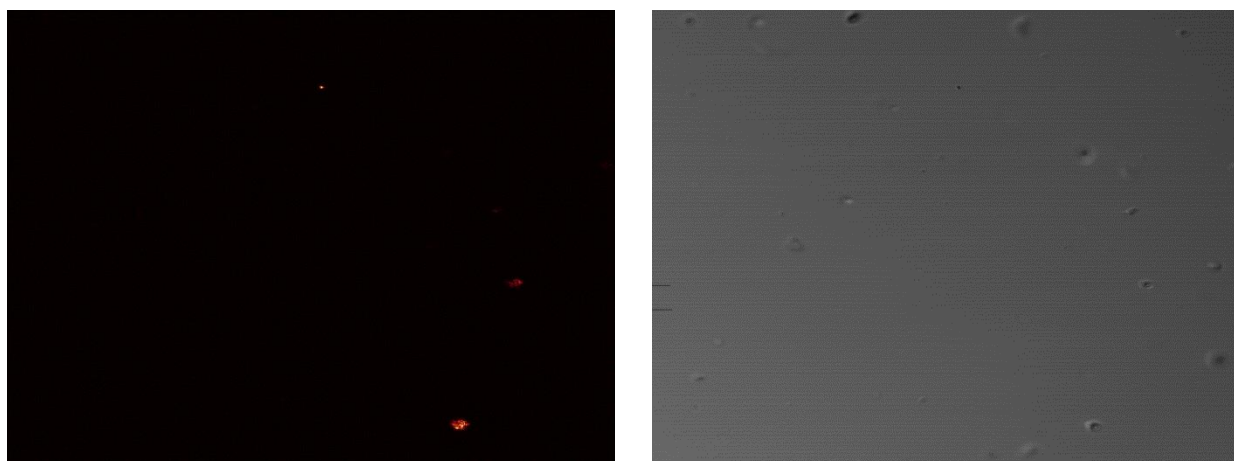
3.1 Silver Nanoparticle Uptake

Table 3.1 Percent of liposomes showing SNP uptake

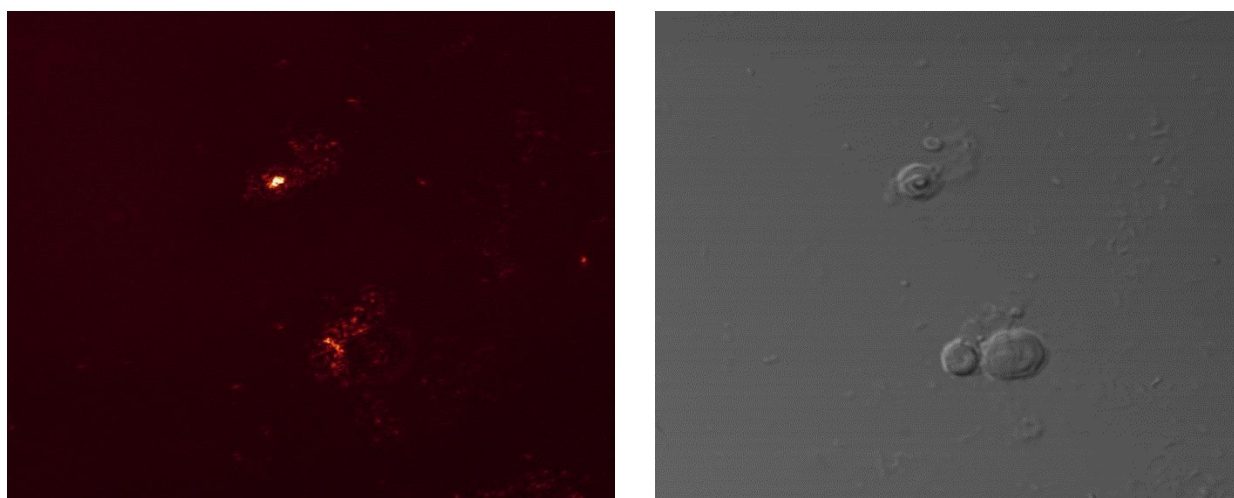
Time (min)	18PC & 18PG	16PC & 16PG	16PC \$ 18PG	16PG \$ 18PC
0	0	0	0	0
30	24.85	53	36	42.4
60	40	63	46	52
120	45.45	73	52	61
240	52.17	92	59	67

Liposomes prepared from 16PC & 16PG phospholipids showed highest amount of uptake and the lowest uptake was observed for 18PC & 18PG. The second highest uptake was for 16PG \$ 18PC followed by 16PC \$ 18PG. Liposomal uptake was not a linear function of time. The rate of uptake was high at the beginning and later the rate slowed down.

Some images are given below showing the uptake of silver nanoparticles by liposomes for different types of phospholipids combinations. Transmission and reflection images are given side by side to make comparison. Left side images are in reflection mode and right side images are taken in transmittance mode of the microscope.

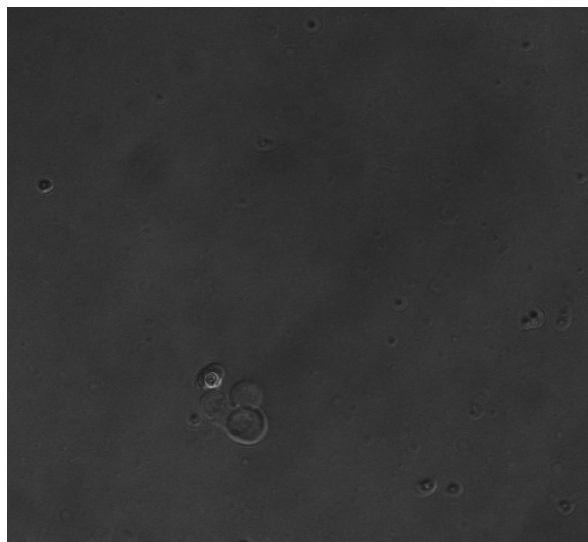


A: Image after 4 hour incubation (18 PC & 18 PG)

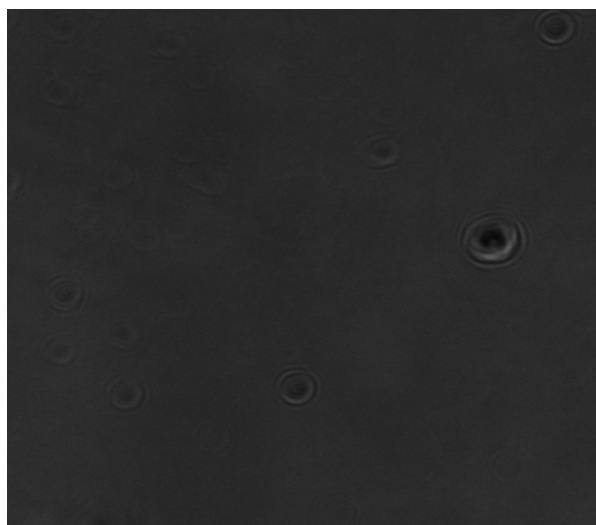
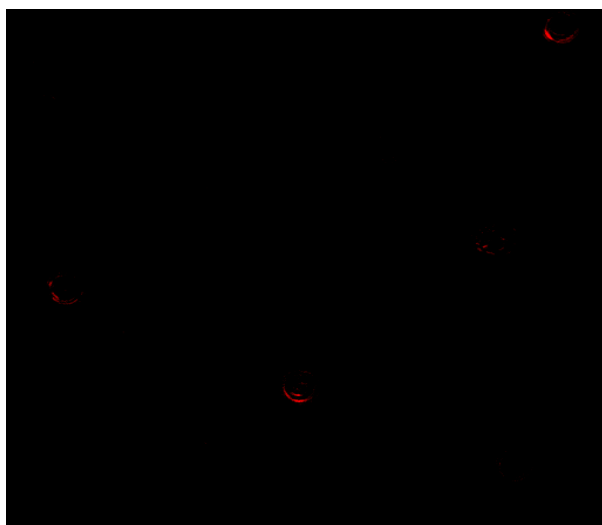


B: Image after 4 hour incubation (16 PC & 16 PG)

Fig 3.1 (A-B) SNP Uptake Images after four hour incubation



C: Image after 4 hour incubation (16 PC & 18 PG)



D: Image after 4 hour incubation (16 PG & 18 PC)

Fig 3.1 (C-D) SNP Uptake Images after four hour incubation

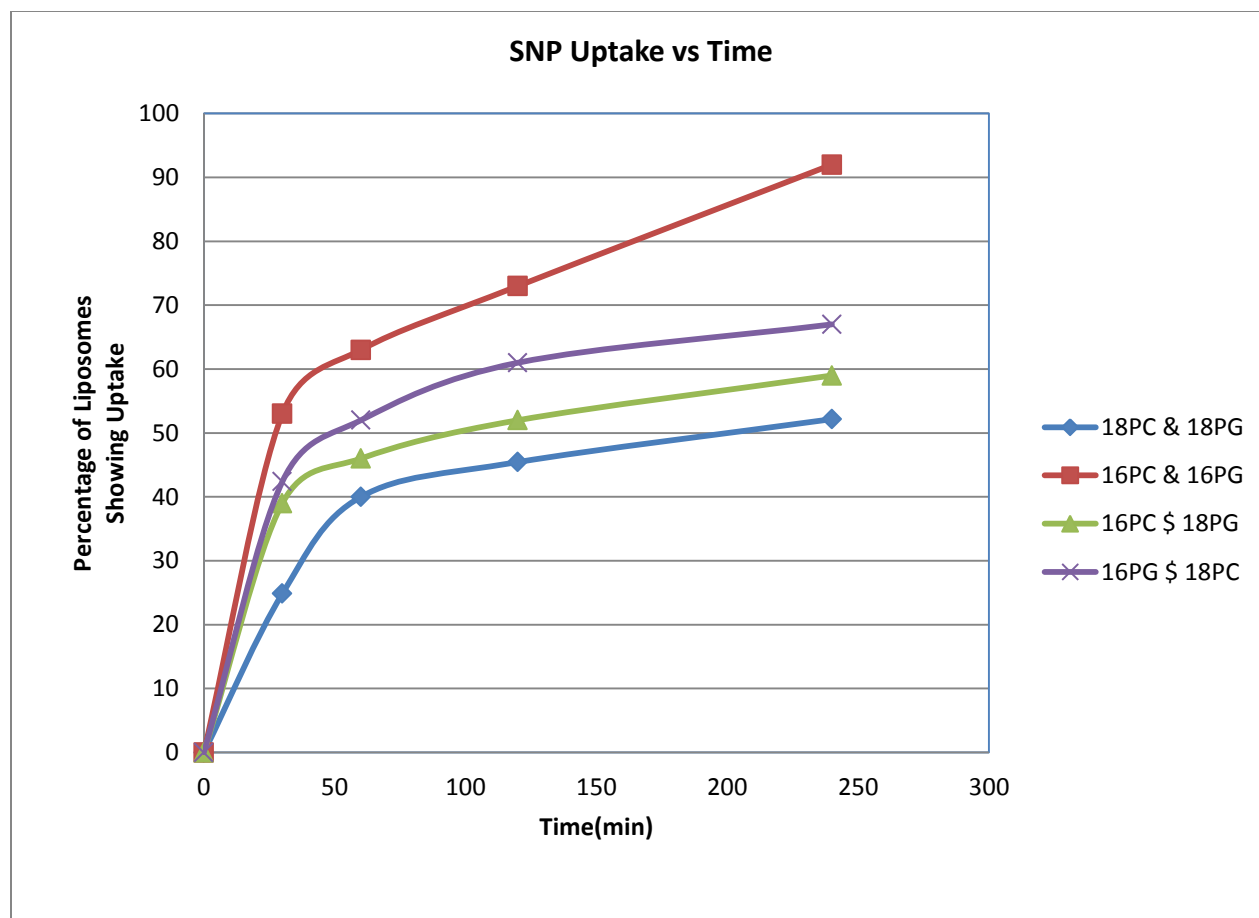


Fig 3.2 Silver Nanoparticle Uptake by Liposomes

The graph shows the uptake of silver nanoparticles by liposomes prepared from four different combinations of phospholipids. For all combinations the uptake rate is high at the beginning which decreases over time. The combination containing shorter alkyl chain phospholipids (16 PC & 16 PG) shows highest amount of uptake and the combination larger alkyl chain length phospholipids (18 PC & 18 PG) showed lowest amount of uptake. Other two combinations showed uptake values in between.

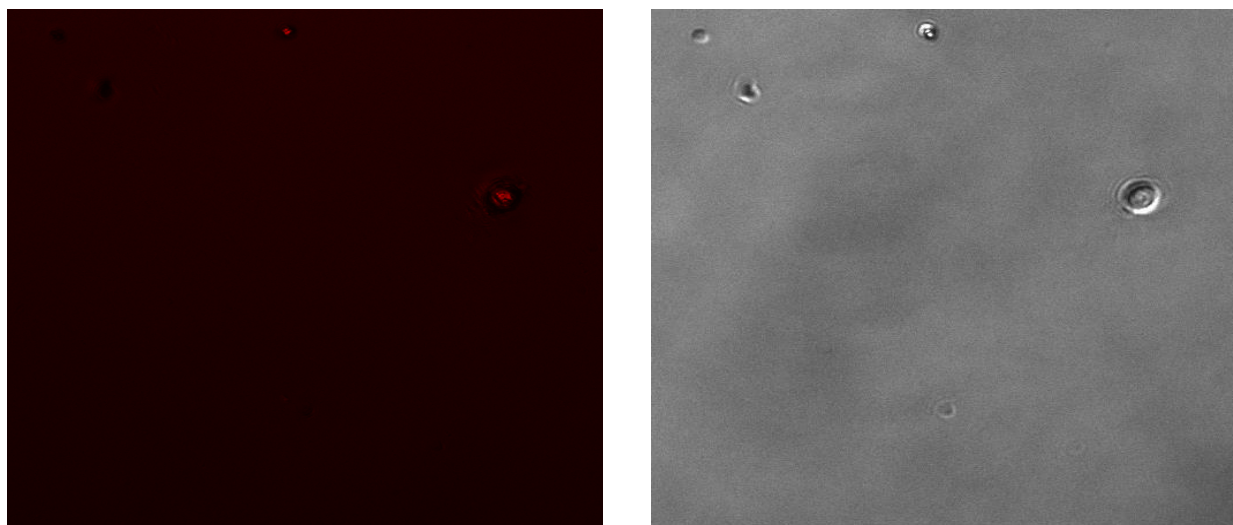
3.2 Gold Nanoparticle Uptake

Table 3.2 Percent of liposomes showing GNP uptake

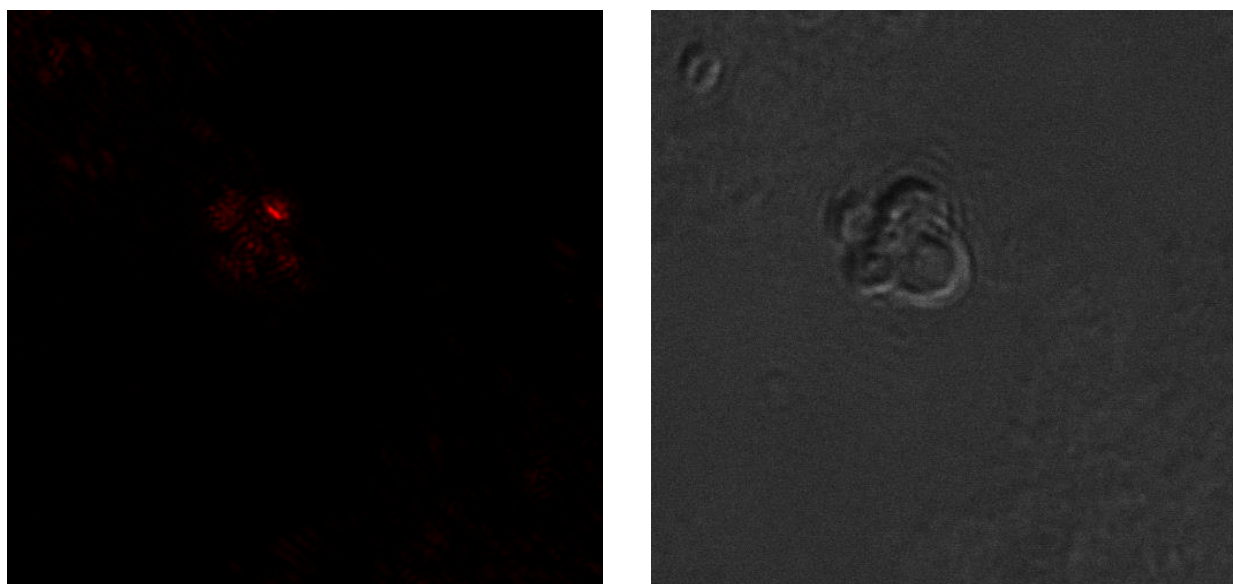
Time (min)	18 PC & 18 PG	16 PC \$ 16 PG	16PC \$ 18PG	16PG \$ 18PC
0	0	0	0	0
30	21	46.8	30.76	35.7
60	34.8	56.2	40	45.8
120	42.85	64.7	47.82	53.6
240	45	72.2	52.17	62.5

The results show similar pattern as obtained from silver nanoparticles. The percentage of liposomes showing uptake was lower than that of silver nanoparticles. Maximum uptake was 72.2% for 16 PC \$ 16 PG and for 18 PC & 18 PG the uptake was 45% after four hours of incubation. The other two combinations showed some value in between.

Similar images were obtained for the liposomal uptake of gold nanoparticles. Some images are given below showing gold nanoparticle uptake.

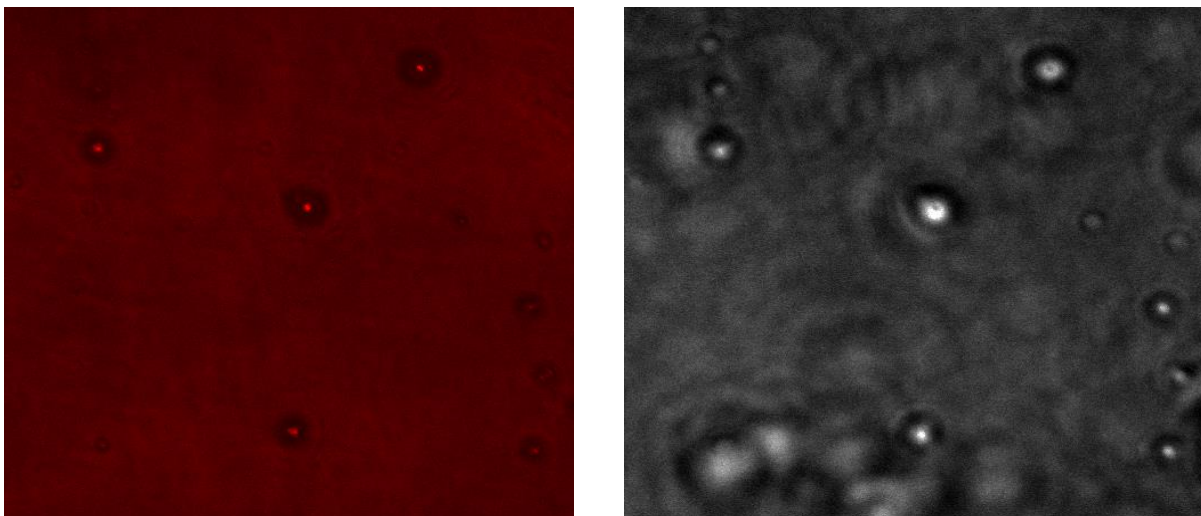


A: Image after 4 hour incubation (18 PC & 18 PG)

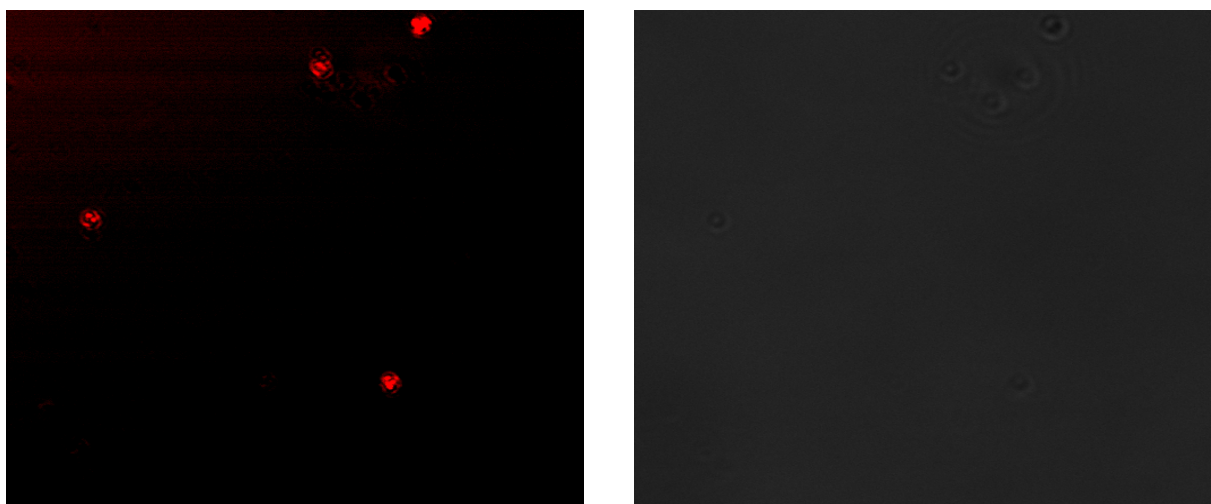


B: Image after 4 hour incubation (16 PC & 16 PG)

Fig 3.3 (A-B) GNP Uptake Images after four hour incubation



C: Image after 4 hour incubation (16 PC & 18 PG)



D: Image after 4 hour incubation (16 PG & 18 PC)

Fig 3.3 (C-D) GNP Uptake Images after four hour incubation

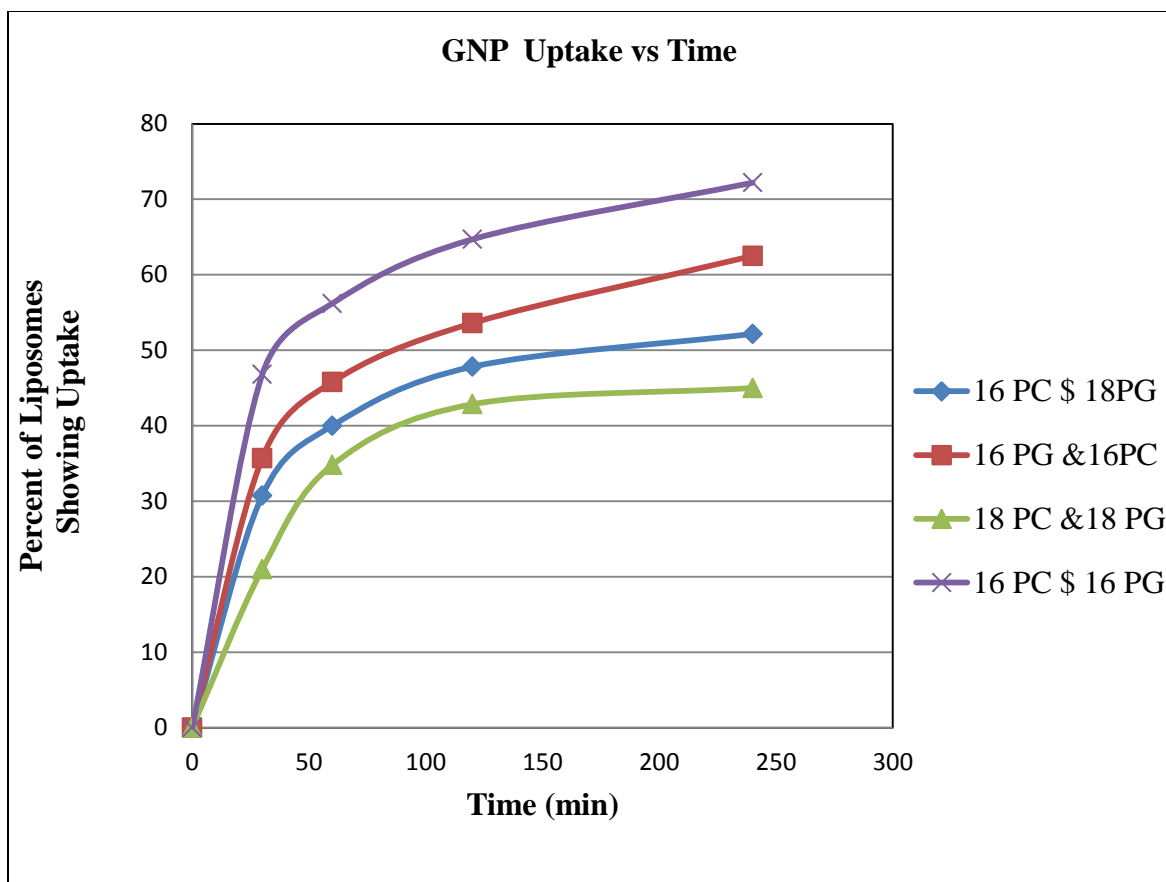
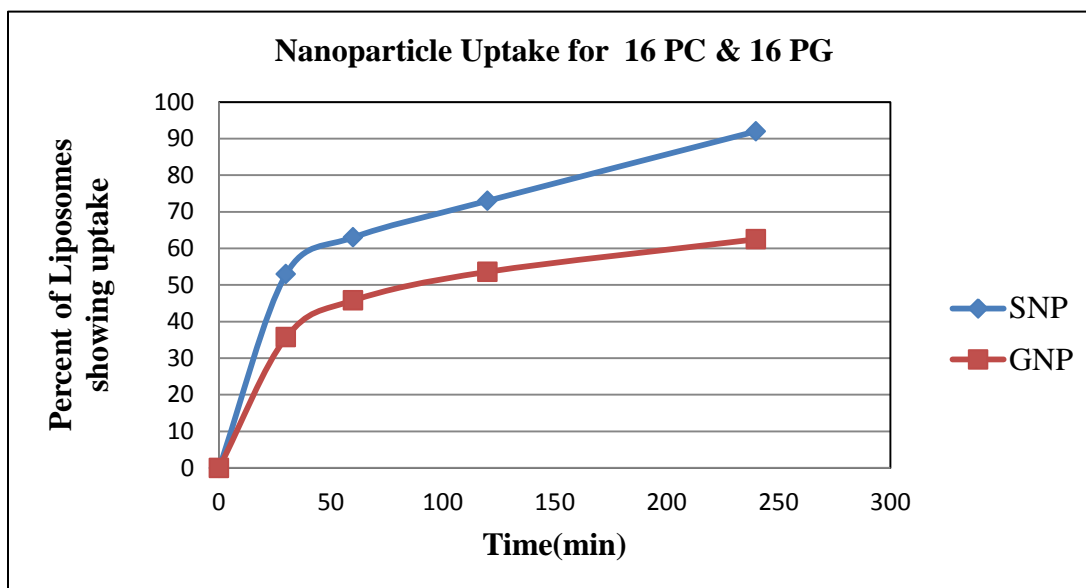


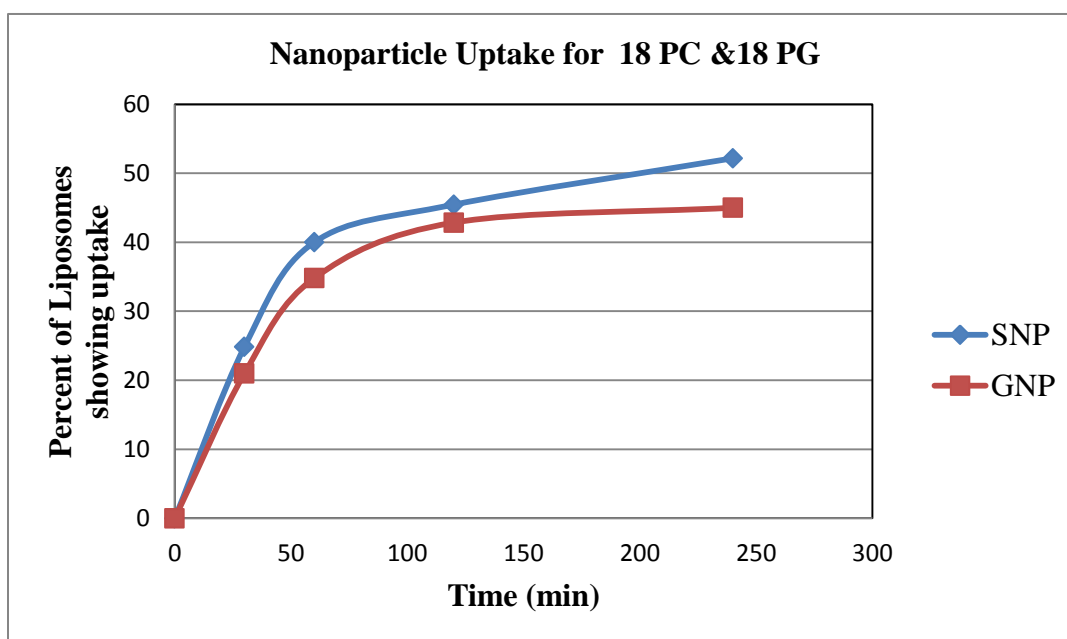
Fig 3.4 Gold Nanoparticle Uptake by Liposomes

The graph shows the percent of liposomes showing gold nanoparticle uptake for four different types of liposomes. The pattern is similar to the uptake by silver nanoparticles. The corresponding values of uptake are lower than that of silver nanoparticles. Highest uptake was observed for 16 PC & 16 PG liposomes and lowest uptake was observed for 18 PC and 18 PG liposomes. The other two combinations of phospholipids showed uptake values in between.

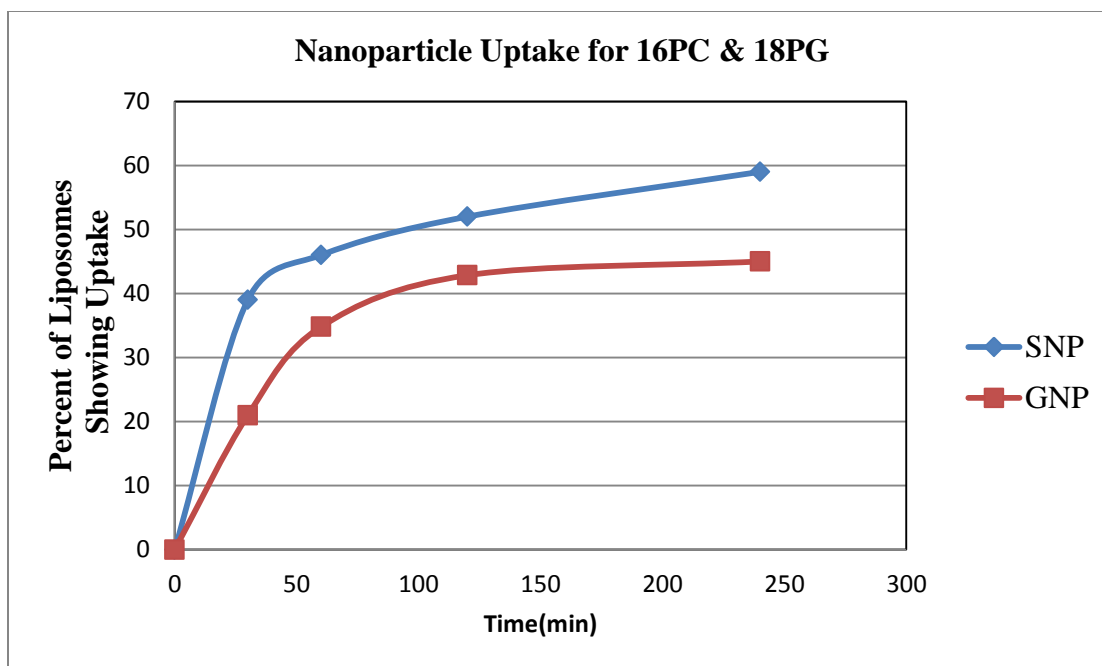
3.3 Comparison between SNP and GNP uptake



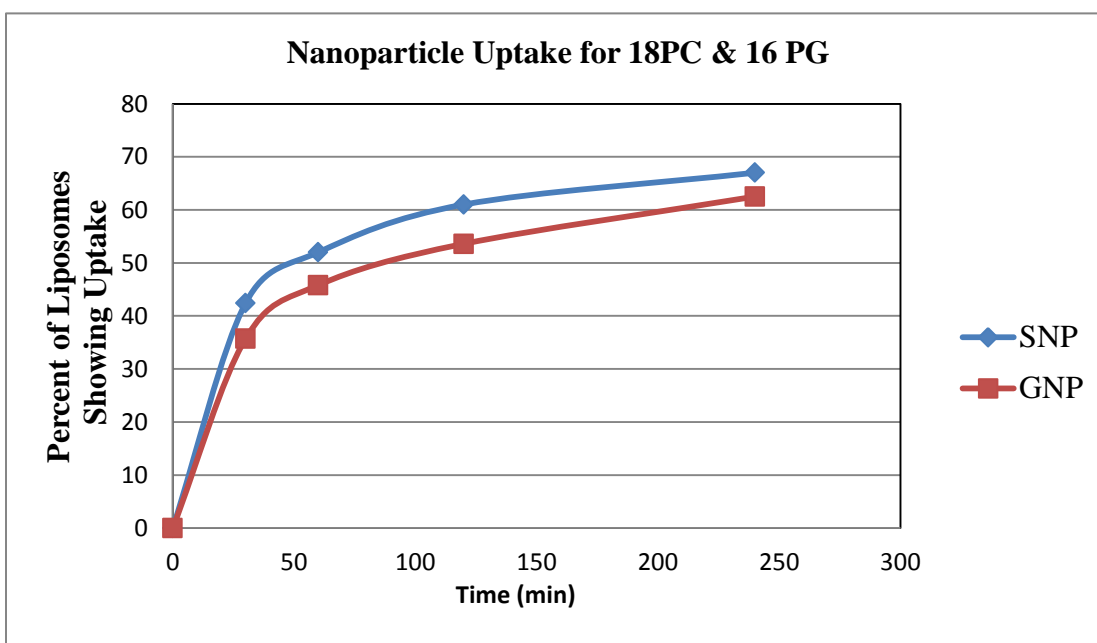
A: Nanoparticle uptake for 16PC & 16PG Liposomes



B: Nanoparticle uptake for 18PC & 18PG Liposomes
Fig 3.5 (A-B) Comparison between SNP and GNP uptake



C: Nanoparticle uptake for 16PC & 18PG Liposomes



D: Nanoparticle uptake for 18PC & 16PG Liposomes

Fig 3.5 (C-D) Comparison between SNP and GNP uptake

The above graphs show the comparison of uptake between silver and gold nanoparticles. In all cases the uptake was higher for silver nanoparticles. The difference in uptake value was maximum for 16 PC & 16 PG liposomes and minimum for 18 PC & 18 PG liposomes. Second highest difference was for 16 PC & 18 PG followed by 16 PG and 18 PC liposomes. The concentration of SNP was 0.25 mg/mL which was much higher than the GNP concentration (10mg/L).

Comparing the magnitude of the concentration of nanoparticles it can be said that the uptake depends more on phospholipids type than the concentration of nanoparticles. There is some obvious relationship between alkyl chain length of the phospholipids and the amount of uptake. Liposomes prepared from phospholipids having 16 carbons in alkyl chain length might be a good carrier for silver and gold nanoparticles of size 60-80nm.

Silver and gold nanoparticles have previously been used to make drug delivery carrier [64-67]. They can be used as imaging and curing agent at the same time [67]. The results obtained in these experiments might be useful for the future development of liposome encapsulated silver and gold nanoparticle based drug delivery system.

3.4 Uncertainty Analysis

Let, P = Actual proportion of liposomes with nanoparticle uptake

p = Proportion of liposomes showing uptake in the sample

n = Sample size or number of liposomes studied

N = Total number of liposomes

Then the estimated variance of p will be $\frac{p(1-p)}{n-1} (1 - \frac{n}{N})$

If 'N' is much larger than 'n' then estimated variance is $\frac{p(1-p)}{n-1}$

The estimated standard error 'S' is the square root of the estimated variance

$$S = \sqrt{\frac{p(1-p)}{n-1}}$$

If p=0.5

$$n=26$$

$$\text{Then } S = \sqrt{\frac{p(1-p)}{n-1}}$$

$$=0.1$$

Table 3.3 Estimated standard error in calculating SNP uptake

Time (min)	18PC & 18PG	16PC & 16PG	16PC \$ 18PG	16PG \$ 18PC
30	0.0415	0.0843	0.0876	0.0873
60	0.1000	0.1053	0.1438	0.1019
120	0.1086	0.0772	0.0944	0.1182
240	0.1064	0.0783	0.0819	0.0980

Table 3.4 Estimated standard error in calculating GNP uptake

Time (min)	18PC & 18PG	16PC & 16PG	16PC \$ 18PG	16PG \$ 18PC
30	0.0960	0.0896	0.0922	0.1328
60	0.1015	0.1281	0.0909	0.1038
120	0.1106	0.1194	0.1064	0.0959
240	0.1141	0.1086	0.1065	0.1250

Although the estimated standard error is a bit high, the actual proportion of liposome having uptake will be very close to the measured value because the images were almost similar all over the slide. If the number of liposomes considered for analysis is increased the standard error will decrease but the proportion of uptake will not change much.

4. CONCLUSION AND FUTURE WORK

The objective of this study was to study the uptake of silver and gold nanoparticles by liposomes prepared from different types of phospholipids. Four different combinations of phospholipids were used to prepare liposomes. The liposomes were incubated with nanoparticle solution for four different incubation periods on a streptavidin coated glass slide. A biotinylated lipid was used for all types of liposomes prepared. The high binding affinity between streptavidin and biotin was used to attach the liposomes on glass slide. After the incubation period the slides were washed to wash off unbound liposomes and nanoparticles. Later confocal microscopy was used to measure the uptake of liposomes by the nanoparticles. The uptake was not a linear function of time. At the beginning the rate of uptake was higher which then decreased over time. The uptake was substantially affected by the type of liposomes used. The maximum uptake was obtained for 16 PC & 16 PG liposomes. Uptake was the minimum for 18 PC & 18 PG liposomes. Uptake was slightly higher for silver nanoparticles than for gold nanoparticles although the concentration was much higher for silver nanoparticles. This suggests the uptake is mainly dependent on the phospholipids types used to prepare liposomes. The relationship between nanoparticle concentration and uptake is not much strong. An important parameter which was not studied is the size of the nanoparticles which might have significant impact on the uptake phenomena. Future researchers may look on this side and can find the optimal nanoparticle size for uptake. The results found in this study might be useful to make liposomes encapsulated nanoparticle based drug delivery carrier.

REFERENCES

1. Maherani, B., Arab-Tehrany, E., R Mozafari, M., Gaiani, C., & Linder, M. (2011). Liposomes: a review of manufacturing techniques and targeting strategies. *Current Nanoscience*, 7(3), 436-452.
2. Meure, L. A., Foster, N. R., & Dehghani, F. (2008). Conventional and dense gas techniques for the production of liposomes: a review. *Aaps Pharmscitech*, 9(3), 798-809.
3. Riaz, M. (1996). Liposomes preparation methods. *Pakistan journal of pharmaceutical sciences*, 19(1), 65-77.
4. Silva, R., Ferreira, H., Little, C., & Cavaco-Paulo, A. (2010). Effect of ultrasound parameters for unilamellar liposome preparation. *Ultrasonics sonochemistry*, 17(3), 628-632.
5. Deamer, D. W. (1978). PREPARATION AND PROPERTIES OF ETHER-INJECTION LIPOSOMES*. *Annals of the New York Academy of Sciences*, 308(1), 250-258.
6. Hauschild, S., Lipprandt, U., Rumpelcker, A., Borchert, U., Rank, A., Schubert, R., & Förster, S. (2005). Direct preparation and loading of lipid and polymer vesicles using inkjets. *Small*, 1(12), 1177-1180.
7. Szoka Jr, F., & Papahadjopoulos, D. (1978). Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. *Proceedings of the National Academy of Sciences*, 75(9), 4194-4198.
8. Jahn, A., Vreeland, W. N., DeVoe, D. L., Locascio, L. E., & Gaitan, M. (2007). Microfluidic directed formation of liposomes of controlled size. *Langmuir*, 23(11), 6289-6293.
9. Sakai, H., Gotoh, T., Imura, T., Sakai, K., Otake, K., & Abe, M. (2008). Preparation and properties of liposomes composed of various phospholipids with different hydrophobic chains using a supercritical reverse phase evaporation method. *Journal of Oleo Science*, 57(11), 613-621.
10. Aburai, K., Yagi, N., Yokoyama, Y., Okuno, H., Sakai, K., Sakai, H., ... & Abe, M. (2011). Preparation of liposomes modified with lipopeptides using a supercritical carbon dioxide reverse-phase evaporation method. *Journal of oleo science*, 60(5), 209.
11. Otake, K., Shimomura, T., Goto, T., Imura, T., Furuya, T., Yoda, S., ... & Abe, M. (2006). Preparation of liposomes using an improved supercritical reverse phase evaporation method. *Langmuir*, 22(6), 2543-2550.

12. Barnadas-Rodríguez, R., & Sabés, M. (2001). Factors involved in the production of liposomes with a high-pressure homogenizer. *International journal of pharmaceutics*, 213(1), 175-186.
13. Hope, M. J., Bally, M. B., Webb, G., & Cullis, P. R. (1985). Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 812(1), 55-65.
14. Mayer, L. D., Hope, M. J., & Cullis, P. R. (1986). Vesicles of variable sizes produced by a rapid extrusion procedure. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 858(1), 161-168.
15. Schneider, T., Sachse, A., Rößling, G., & Brandl, M. (1995). Generation of contrast-carrying liposomes of defined size with a new continuous high pressure extrusion method. *International journal of pharmaceutics*, 117(1), 1-12.
16. Massing, U., Cicko, S., & Ziroli, V. (2008). Dual asymmetric centrifugation (DAC)—A new technique for liposome preparation. *Journal of Controlled Release*, 125(1), 16-24.
17. Hirsch, M., Ziroli, V., Helm, M., & Massing, U. (2009). Preparation of small amounts of sterile siRNA-liposomes with high entrapping efficiency by dual asymmetric centrifugation (DAC). *Journal of Controlled Release*, 135(1), 80-88.
18. Mozafari, M. R. (2005). Liposomes: an overview of manufacturing techniques. *Cellular and Molecular Biology Letters*, 10(4), 711.
19. Mozafari, M. R., Reed, C. J., Rostron, C. H. R. I. S. T. O. P. H. E. R., Kocum, C. E. N. G. I. Z., & Piskin, E. R. H. A. N. (2002). Construction of stable anionic liposome-plasmid particles using the heating method: a preliminary investigation. *Cellular and Molecular Biology Letters*, 7(3), 923-928.
20. Mortazavi, S. M., Mohammadabadi, M. R., Khosravi-Darani, K., & Mozafari, M. R. (2007). Preparation of liposomal gene therapy vectors by a scalable method without using volatile solvents or detergents. *Journal of biotechnology*, 129(4), 604-613.
21. Genç, R., Ortiz, M., & O' Sullivan, C. K. (2009). Curvature-Tuned preparation of nanoliposomes. *Langmuir*, 25(21), 12604-12613.
22. Moussaoui, N., Cansell, M., & Denizot, A. (2002). Marinosomes®, marine lipid-based liposomes: physical characterization and potential application in cosmetics. *International journal of pharmaceutics*, 242(1), 361-365.

23. Betz, G., Aeppli, A., Menshutina, N., & Leuenberger, H. (2005). In vivo comparison of various liposome formulations for cosmetic application. *International journal of pharmaceutics*, 296(1), 44-54.
24. Benech, R. O., Kheadr, E. E., Laridi, R., Lacroix, C., & Fliss, I. (2002). Inhibition of *Listeria innocua* in cheddar cheese by addition of nisin Z in liposomes or by in situ production in mixed culture. *Applied and environmental microbiology*, 68(8), 3683-3690.
25. Kheadr, E. E., Vuillemand, J. C., & El-Deeb, S. A. (2002). Acceleration of Cheddar Cheese Lipolysis by Using Liposome-entrapped Lipases. *Journal of food science*, 67(2), 485-492.
26. Kheadr, E. E., Vuillemand, J. C., & El Deeb, S. A. (2000). Accelerated Cheddar cheese ripening with encapsulated proteinases. *International journal of food science & technology*, 35(5), 483-495.
27. Ding, B., Xia, S., Hayat, K., & Zhang, X. (2009). Preparation and pH stability of ferrous glycinate liposomes. *Journal of agricultural and food chemistry*, 57(7), 2938-2944.
28. Xia, S., & Xu, S. (2005). Ferrous sulfate liposomes: preparation, stability and application in fluid milk. *Food research international*, 38(3), 289-296.
29. Nacka, F., Cansell, M., Méléard, P., & Combe, N. (2001). Incorporation of α -tocopherol in marine lipid-based liposomes: in vitro and in vivo studies. *Lipids*, 36(12), 1313-1320.
30. Rao, D. R., Chawan, C. B., & Veeramachaneni, R. (1994). LIPOSOMAL ENCAPSULATION OF β -GALACTOSIDASE: COMPARISON OF TWO METHODS OF ENCAPSULATION AND IN VITRO LACTOSE DIGESTIBILITY. *Journal of food biochemistry*, 18(4), 239-251.
31. Albaldawi, A., Brennan, C. S., Alobaidy, K., Alammari, W., & Aljumaily, D. (2005). Effect of flour fortification with haem liposome on bread and bread doughs. *International journal of food science & technology*, 40(8), 825-828.
32. Colas, J. C., Shi, W., Rao, V. S. N., Omri, A., Mozafari, M. R., & Singh, H. (2007). Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. *Micron*, 38(8), 841-847.
33. Panwar, P., Pandey, B., Lakhera, P. C., & Singh, K. P. (2010). Preparation, characterization, and in vitro release study of albendazole-encapsulated nanosize liposomes. *International journal of nanomedicine*, 5, 101.

34. Wang, Y., Tu, S., Li, R., Yang, X., Liu, L., & Zhang, Q. (2010). Cholesterol succinyl chitosan anchored liposomes: preparation, characterization, physical stability, and drug release behavior. *Nanomedicine: Nanotechnology, Biology and Medicine*, 6(3), 471-477.
35. Xiang, Q., Xiao, J., Zhang, H., Zhang, X., Lu, M., Zhang, H., ... & Li, X. (2011). Preparation and characterisation of bFGF-encapsulated liposomes and evaluation of wound-healing activities in the rat. *Burns*, 37(5), 886-895.
36. Shah, S. P., & Misra, A. (2004). Liposomal amikacin dry powder inhaler: effect of fines on in vitro performance. *AAPS PharmSciTech*, 5(4), 107-113.
37. Niu, M., Lu, Y., Hovgaard, L., & Wu, W. (2011). Liposomes containing glycocholate as potential oral insulin delivery systems: preparation, in vitro characterization, and improved protection against enzymatic degradation. *International journal of nanomedicine*, 6, 1155.
38. Maruyama, K., Ishida, O., Kasaoka, S., Takizawa, T., Utoguchi, N., Shinohara, A., ... & Yanagie, H. (2004). Intracellular targeting of sodium mercaptoundecahydrododecaborate (BSH) to solid tumors by transferrin-PEG liposomes, for boron neutron-capture therapy (BNCT). *Journal of controlled release*, 98(2), 195-207.
39. Koudelka, Š., Turánek-Knötigová, P., Mašek, J., Korvasová, Z., Škrabalová, M., Plocková, J., ... & Turánek, J. (2010). Liposomes with high encapsulation capacity for paclitaxel: preparation, characterisation and in vivo anticancer effect. *Journal of pharmaceutical sciences*, 99(5), 2309-2319.
40. Takeuchi, H., Kojima, H., Yamamoto, H., & Kawashima, Y. (2001). Passive targeting of doxorubicin with polymer coated liposomes in tumor bearing rats. *Biological and Pharmaceutical Bulletin*, 24(7), 795-799.
41. Layek, B., & Mukherjee, B. (2010). Tamoxifen citrate encapsulated sustained release liposomes: preparation and evaluation of physicochemical properties. *Scientia pharmaceutica*, 78(3), 507.
42. Liang, X. F., Wang, H. J., Luo, H., Tian, H., Zhang, B. B., Hao, L. J., ... & Chang, J. (2008). Characterization of novel multifunctional cationic polymeric liposomes formed from octadecyl quaternized carboxymethyl chitosan/cholesterol and drug encapsulation. *Langmuir*, 24(14), 7147-7153.
43. Fountain, M. W., Dees, C., & Schultz, R. D. (1981). Enhanced intracellular killing of *Staphylococcus aureus* by canine monocytes treated with liposomes containing amikacin, gentamicin, kanamycin, and tobramycin. *Current Microbiology*, 6(6), 373-376.

44. Mizoue, T., Horibe, T., Maruyama, K., Takizawa, T., Iwatsuru, M., Kono, K., ... & Moriyasu, F. (2002). Targetability and intracellular delivery of anti-BCG antibody-modified, pH-sensitive fusogenic immunoliposomes to tumor cells. *International journal of pharmaceutics*, 237(1), 129-137.
45. Babincova, M., Sourivong, P., Chorvát, D., & Babinec, P. (1999). Laser triggered drug release from magnetoliposomes. *Journal of magnetism and magnetic materials*, 194(1), 163-166.
46. Han, H. D., Kim, T. W., Shin, B. C., & Choi, H. S. (2005). Release of calcein from temperature-sensitive liposomes. *Macromolecular Research*, 13(1), 54-61.
47. Ögün Çirli, Ö., & Hasırcı, V. (2004). UV-induced drug release from photoactive REV sensitized by suprofen. *Journal of controlled release*, 96(1), 85-96.
48. Zheng, X., Lu, J., Deng, L., Xiong, Y., & Chen, J. (2009). Preparation and characterization of magnetic cationic liposome in gene delivery. *International Journal of Pharmaceutics*, 366(1), 211-217.
49. Lesoin, L., Crampon, C., Boutin, O., & Badens, E. (2011). Preparation of liposomes using the supercritical anti-solvent (SAS) process and comparison with a conventional method. *The Journal of Supercritical Fluids*, 57(2), 162-174.
50. Liang, X., Wang, H., Jiang, X., & Chang, J. (2010). Development of monodispersed and functional magnetic polymeric liposomes via simple liposome method. *Journal of Nanoparticle Research*, 12(5), 1723-1732.
51. Berger, N., Sachse, A., Bender, J., Schubert, R., & Brandl, M. (2001). Filter extrusion of liposomes using different devices: comparison of liposome size, encapsulation efficiency, and process characteristics. *International journal of pharmaceutics*, 223(1), 55-68.
52. Otake, K., Imura, T., Sakai, H., & Abe, M. (2001). Development of a new preparation method of liposomes using supercritical carbon dioxide. *Langmuir*, 17(13), 3898-3901.
53. Khaleque, M. A., Okumura, Y., Yabushita, S., & Mitani, M. (2004). Detachable immobilization of liposomes on polymer gel particles. *Colloids and Surfaces B: Biointerfaces*, 37(1), 35-42.
54. Liu, X. Y., Yang, Q., Nakamura, C., & Miyake, J. (2001). Avidin-biotin-immobilized liposome column for chromatographic fluorescence on-line analysis of solute-membrane interactions. *Journal of Chromatography B: Biomedical Sciences and Applications*, 750(1), 51-60.

55. Ma, Y., Zhang, H., & Sun, X. L. (2010). Surface-bound cytomimetic assembly based on chemoselective and biocompatible immobilization and further modification of intact liposome. *Bioconjugate chemistry*, 21(11), 1994-1999.
56. Kalyankar, N. D., Sharma, M. K., Vaidya, S. V., Calhoun, D., Maldarelli, C., Couzis, A., & Gilchrist, L. (2006). Arraying of intact liposomes into chemically functionalized microwells. *Langmuir*, 22(12), 5403-5411.
57. Pignataro, B., Steinem, C., Galla, H. J., Fuchs, H., & Janshoff, A. (2000). Specific adhesion of vesicles monitored by scanning force microscopy and quartz crystal microbalance. *Biophysical journal*, 78(1), 487-498.
58. Jung, L. S., Shumaker-Parry, J. S., Campbell, C. T., Yee, S. S., & Gelb, M. H. (2000). Quantification of tight binding to surface-immobilized phospholipid vesicles using surface plasmon resonance: binding constant of phospholipase A2. *Journal of the American Chemical Society*, 122(17), 4177-4184.
59. Losey, E. A., Smith, M. D., Meng, M., & Best, M. D. (2009). Microplate-Based Analysis of Protein– Membrane Binding Interactions via Immobilization of Whole Liposomes Containing a Biotinylated Anchor. *Bioconjugate chemistry*, 20(2), 376-383.
60. Rongen, H. A. H., Van Nierop, T., Van der Horst, H. M., Rombouts, R. F. M., Van der Meide, P. H., Bult, A., & Van Bennekom, W. P. (1995). Biotinylated and streptavidinylated liposomes as labels in cytokine immunoassays. *Analytica chimica acta*, 306(2), 333-341.
61. Smith, M. D., & Best, M. D. (2011). Characterization of Protein–Membrane Binding Interactions via a Microplate Assay Employing Whole Liposome Immobilization. In *Bioconjugation Protocols* (pp. 477-489). Humana Press.
62. Vermette, P., Meagher, L., Gagnon, E., Griesser, H. J., & Doillon, C. J. (2002). Immobilized liposome layers for drug delivery applications: inhibition of angiogenesis. *Journal of controlled release*, 80(1), 179-195.
63. Vermette, P., Griesser, H. J., Kambouris, P., & Meagher, L. (2004). Characterization of surface-immobilized layers of intact liposomes. *Biomacromolecules*, 5(4), 1496-1502.
64. Rayavarapu, R. G., Petersen, W., Ungureanu, C., Post, J. N., van Leeuwen, T. G., & Manohar, S. (2007). Synthesis and bioconjugation of gold nanoparticles as potential molecular probes for light-based imaging techniques. *Journal of Biomedical Imaging*, 2007(1), 5-5.
65. Tsai, S. W., Chen, Y. Y., & Liaw, J. W. (2008). Compound cellular imaging of laser scanning confocal microscopy by using gold nanoparticles and dyes. *Sensors*, 8(4), 2306-2316.

66. Brown, P. K., Qureshi, A. T., Moll, A. N., Hayes, D. J., & Monroe, W. T. (2013). Silver Nanoscale Antisense Drug Delivery System for Photoactivated Gene Silencing. *ACS nano*.
67. Sokolov, K., Follen, M., Aaron, J., Pavlova, I., Malpica, A., Lotan, R., & Richards-Kortum, R. (2003). Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Research*, 63(9), 1999-2004.

VITA

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